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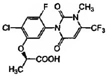
MARKS & CLERK

(54) PROTEINES DU METABOLISME PERMETTANT DE LUTTER CONTRE LES MAUVAISES HERBES, LEURS GENES ET LEUR UTILISATION

(54) WEED CONTROLLER METABOLISM PROTEINS, GENES THEREOF AND USE OF THE SAME

(57)
It is intended to provide DNAs encoding a weed controller metabolism protein selected from the following group, etc. which are useful in, for example, constructing a weed controller-tolerant plant. <Proteins> Proteins having an amino acid sequence represented by SEQ ID NO: 1, 2, 3, 108, 159, 160, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224. Proteins having an amino acid sequence showing a sequence identity of 80% or more with one of the amino acid sequences represented by SEQ ID NOS: 1, 2, 3, 108, 159, 136, 137, 138, 217, 219, 220, 221 and 223, or an amino acid sequence showing a sequence identity of 90% or more with one of the amino acid sequences represented by SEQ ID NO: 160, 215, 216, 218, 222 and 224, and being capable of converting the compound represented by the following formula (II): (II) into the compound represented by the following formula (III): (III) in the presence of an electron transfer system from an electron donor.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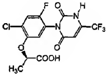
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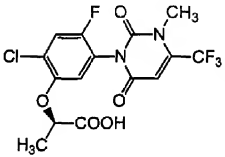
(54) Titre : PROTEINES DU METABOLISME PERMETTANT DE LUTTER CONTRE LES MAUVAISES HERBES, LEURS GENES ET LEUR UTILISATION
(54) Title: WEED CONTROLLER METABOLISM PROTEINS, GENES THEREOF AND USE OF THE SAME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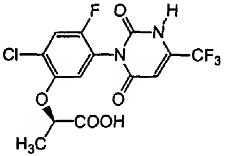

(III)

(57) Abrégé/Abstract:
It is intended to provide DNAs encoding a weed controller metabolism protein selected from the following group, etc. which are useful in, for example, constructing a weed controller-tolerant plant. <Proteins> Proteins having an amino acid sequence represented by SEQ ID NO. 1, 2, 3, 108, 159, 160, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224. Proteins having an amino acid sequence showing a sequence identity of 80% or more with one of the amino acid sequences represented by SEQ ID NOS: 1, 2, 3, 108, 159, 136, 137, 138, 217, 219, 220, 221 and 223, or an amino acid sequence showing a sequence identity of 90% or more with one of the amino acid sequences represented by SEQ ID NO: 160, 215, 216, 218, 222 and 224, and being capable of converting the compound represented by the following formula (II): (II) into the compound represented by the following formula (III): (III) in the presence of an electron transfer system from an electron donor.

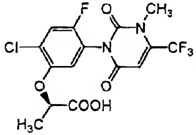
ABSTRACT

The present invention provides, for example, DNA encoding a herbicide metabolizing protein selected from the protein group below. Such DNA may, for example, be employed to produce herbicidally resistant plants.

<protein group>

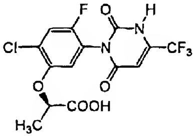
a protein comprising the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 108, 159, 160, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224,

a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II):



(II)

to a compound of formula (III):



(III)

and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, 2, 3, 108, 159, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID

NO: 160, 215, 216, 218, 222 or 224.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 349

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 349

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NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

DESCRIPTION

A HERBICIDE METABOLIZING PROTEIN, A GENE THEREOF AND USE THEREOF

TECHNICAL FIELD

The present invention relates to a protein having the ability to metabolize a herbicidal compound (Herbicide metabolizing protein), a gene thereof and use thereof.

BACKGROUND ART

Herbicides are utilized in a necessary amount of diluted solution when applied. There are situations in which extra amounts are left over. There are also situations in which the applied herbicide, after its application for awhile, remains in the soil or plant residue. Originally, given that the safety of such herbicides has been checked, such small amounts of left-over solutions or residues presented small effects to the environment or to the crops cultivated thereafter. However, if there is a method in which the contained herbicidal compound is converted to one of lower herbicidal activity, then for example there can be conducted treatments to inactivate the left-over solutions or residues described above as needed.

Further, in the case of using the herbicide, there were situations in which it was difficult to distinguish cultivated plants from weeds of allied species to selectively control only weeds. Then, there is a desire to develop a new method for conferring herbicidal resistance to a target plant.

DISCLOSURE OF THE INVENTION

Under such the circumstances, the present inventors intensively studied and, as a result, have found that a protoporphyrinogen oxidase (hereinafter, sometimes referred to as "PPO") inhibitory-type herbicidal compound may be converted by being reacted with a particular protein to a compound of lower herbicidal activity, which resulted in completion of the present invention.

That is, the present invention provides:

1. A DNA encoding a herbicide metabolizing protein, wherein said protein is selected from the group consisting of:

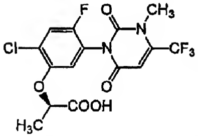
(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

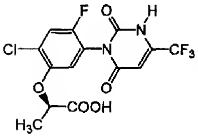
(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II):



(II)

to a compound of formula (III):



(III)

and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport

system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224;

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising the nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseofuscus, Streptomyces thermocoerulescens,

Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis, Streptomyces steffisburgensis or Saccharopolyspora taberi;

2. A DNA comprising a nucleotide sequence selected from the group consisting of:

(a1) the nucleotide sequence shown in SEQ ID NO: 6;

(a2) the nucleotide sequence shown in SEQ ID NO: 7;

(a3) the nucleotide sequence shown in SEQ ID NO: 8;

(a4) the nucleotide sequence shown in SEQ ID NO: 109;

(a5) the nucleotide sequence shown in SEQ ID NO: 139;

(a6) the nucleotide sequence shown in SEQ ID NO: 140;

(a7) the nucleotide sequence shown in SEQ ID NO: 141;

(a8) the nucleotide sequence shown in SEQ ID NO: 142;

(a9) the nucleotide sequence shown in SEQ ID NO: 143;

(a10) the nucleotide sequence shown in SEQ ID NO: 225;

(a11) the nucleotide sequence shown in SEQ ID NO: 226;

(a12) the nucleotide sequence shown in SEQ ID NO: 227;

(a13) the nucleotide sequence shown in SEQ ID NO: 228;

(a14) the nucleotide sequence shown in SEQ ID NO: 229;

(a15) the nucleotide sequence shown in SEQ ID NO: 230;

(a16) the nucleotide sequence shown in SEQ ID NO: 231;

(a17) the nucleotide sequence shown in SEQ ID NO: 232;

(a18) the nucleotide sequence shown in SEQ ID NO: 233;

(a19) the nucleotide sequence shown in SEQ ID NO: 234;

(a20) a nucleotide sequence encoding an amino acid sequence of a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), said nucleotide sequence having at least 80% sequence identity with a nucleotide sequence shown in any one of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 109; and

(a21) a nucleotide sequence encoding an amino acid sequence of a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), said nucleotide sequence having at least 90% sequence identity with a nucleotide sequence shown in any one of SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 225, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233 or SEQ ID NO: 234;

3. The DNA according to the above 1, comprising a nucleotide sequence encoding an amino acid sequence of said protein, wherein the codon usage in said nucleotide sequence is within the range of plus or minus 4% of the codon usage in genes from the species of a host cell to which the DNA is introduced and the GC content of said nucleotide sequence is at least 40% and at most 60%;

4. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 214;

5. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 368;

6. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 393;

7. A DNA in which a DNA having a nucleotide sequence encoding an intracellular organelle transit signal sequence is linked upstream of the DNA according to the above 1 in frame;

8. A DNA in which the DNA according to the above 1 and a promoter functional in a host cell are operably linked.;

9. A vector comprising the DNA according to the above 1;

10. A method of producing a vector comprising a step of inserting the DNA according to the above 1 into a vector replicable in a host cell;

11. A transformant in which the DNA according to the above 1 is introduced into a host cell;

12. The transformant according to the above 11, wherein the host cell is a microorganism cell or a plant cell;

13. A method of producing a transformant comprising a step of introducing into a host cell, the DNA according to the above 1;

14. A method of producing a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method comprising a steps of culturing the transformant according to the above 11 and recovering the produced said protein;

15. Use of the DNA according to the above 1 for producing a protein having the ability to convert a compound of formula (II) to a compound of formula (III);

16. A method of giving a plant resistance to a herbicide, said method comprising a step of introducing into and expressing in a plant cell, the DNA according to the above 1;

17. A polynucleotide having a partial nucleotide sequence of a DNA according to the above 1 or a nucleotide sequence complimentary to said partial nucleotide sequence;

18. A method of detecting a DNA encoding a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method comprising a step of detecting a DNA to which a probe is hybridized in a hybridization using as the probe the DNA according to the above 1 or the polynucleotide according to the above 17;

19. A method of detecting a DNA encoding a protein having the ability to convert a

compound of formula (II) to a compound of formula (III), said method comprising a step of detecting a DNA amplified in a polymerase chain reaction with the polynucleotide according to the above 17 as a primer;

20. The method according to the above 19, wherein at least one of the primers is selected from the group consisting of a polynucleotide comprising the nucleotide sequence shown in any one of SEQ ID NOs:124 to 128 and a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129;

21. A method of obtaining a DNA encoding a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method comprising a step of recovering the DNA detected by the method according to the above 18 or 19.

22. A method of screening a cell having a DNA encoding a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method comprising a step of detecting said DNA from a test cell by the method according to the above 18 or 19;

23. A herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system

containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO:

219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224;

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising the nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseofuscus, Streptomyces thermococrulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis,

Streptomyces steffisburgensis or Saccharopolyspora taberi;

24. An antibody recognizing a herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

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(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

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(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

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(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO:

224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising the nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis, Streptomyces steffisburgensis or Saccharopolyspora taberi;

25. A method of detecting a herbicide metabolizing protein, said method comprising:

(1) a step of contacting a test substance with an antibody recognizing said protein and

(2) a step of detecting a complex of said protein and said antibody, arising from said contact,

wherein said protein is selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system

containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224;

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising the nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces

achromogenes, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis, Streptomyces steffisburgensis or Saccharopolyspora taberi;

26. An analysis or detection kit comprising the antibody according to the above 24;

27. A DNA encoding a ferredoxin selected from the group consisting of:

(B1) a protein comprising an amino acid sequence shown in SEQ ID NO: 12;

(B2) a protein comprising an amino acid sequence shown in SEQ ID NO: 13;

(B3) a protein comprising an amino acid sequence shown in SEQ ID NO: 14;

(B4) a protein comprising an amino acid sequence shown in SEQ ID NO: 111;

(B5) a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

(B6) a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

(B7) a protein comprising an amino acid sequence shown in SEQ ID NO: 149;

(B8) a protein comprising an amino acid sequence shown in SEQ ID NO: 150;

(B9) a protein comprising an amino acid sequence shown in SEQ ID NO: 151;

(B10) a protein comprising an amino acid sequence shown in SEQ ID NO: 152;

(B11) a protein comprising an amino acid sequence shown in SEQ ID NO: 153;

(B12) a ferredoxin comprising an amino acid sequence having at least 80% sequence

identity with an amino acid sequence shown in any one of SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, or SEQ ID NO: 253 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 150, SEQ ID NO: 252 or SEQ ID NO: 254;

(B13) a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253 or SEQ ID NO: 254;

(B14) a protein comprising the amino acid sequence shown in SEQ ID NO: 245;

(B15) a protein comprising the amino acid sequence shown in SEQ ID NO: 247;

(B16) a protein comprising the amino acid sequence shown in SEQ ID NO: 248;

(B17) a protein comprising the amino acid sequence shown in SEQ ID NO: 249;

(B18) a protein comprising the amino acid sequence shown in SEQ ID NO: 250;

(B19) a protein comprising the amino acid sequence shown in SEQ ID NO: 251;

(B20) a protein comprising the amino acid sequence shown in SEQ ID NO: 252;

(B21) a protein comprising the amino acid sequence shown in SEQ ID NO: 253; and

(B22) a protein comprising the amino acid sequence shown in SEQ ID NO: 254;

28. A DNA comprising a nucleotide sequence selected from the group consisting of:

(b1) a nucleotide sequence shown in SEQ ID NO: 15;

(b2) a nucleotide sequence shown in SEQ ID NO: 16;

(b3) a nucleotide sequence shown in SEQ ID NO: 17;

(b4) a nucleotide sequence shown in SEQ ID NO: 112;

(b5) a nucleotide sequence shown in SEQ ID NO: 154;

(b6) a nucleotide sequence shown in SEQ ID NO: 155;

(b7) a nucleotide sequence shown in SEQ ID NO: 156;

(b8) a nucleotide sequence shown in SEQ ID NO: 157;

(b9) a nucleotide sequence shown in SEQ ID NO: 158;

(b10) a nucleotide sequence shown in SEQ ID NO: 255;

(b11) a nucleotide sequence shown in SEQ ID NO: 257;

(b12) a nucleotide sequence shown in SEQ ID NO: 258;

(b13) a nucleotide sequence shown in SEQ ID NO: 259;

(b14) a nucleotide sequence shown in SEQ ID NO: 260;

(b15) a nucleotide sequence shown in SEQ ID NO: 261;

(b16) a nucleotide sequence shown in SEQ ID NO: 262;

(b17) a nucleotide sequence shown in SEQ ID NO: 263;

(b18) a nucleotide sequence shown in SEQ ID NO: 264; and

(b19) a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence shown in any one of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 112, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263 or SEQ ID NO: 264;

29. A vector comprising a DNA according to the above 28;

30. A transformant in which the DNA according to the above 28 is introduced into a host cell;

31. A ferredoxin selected from the group consisting of:

(B1) a protein comprising an amino acid sequence shown in SEQ ID NO: 12;

(B2) a protein comprising an amino acid sequence shown in SEQ ID NO: 13;

(B3) a protein comprising an amino acid sequence shown in SEQ ID NO: 14;

(B4) a protein comprising an amino acid sequence shown in SEQ ID NO: 111;

(B5) a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

(B6) a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

(B7) a protein comprising an amino acid sequence shown in SEQ ID NO: 149;

(B8) a protein comprising an amino acid sequence shown in SEQ ID NO: 150;

(B9) a protein comprising an amino acid sequence shown in SEQ ID NO: 151;

(B10) a protein comprising an amino acid sequence shown in SEQ ID NO: 152;

(B11) a protein comprising an amino acid sequence shown in SEQ ID NO: 153;

(B12) a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, or SEQ ID NO: 253 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 150, SEQ ID NO: 252 or SEQ ID NO: 254;

(B13) a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence

encoding an amino acid sequence shown in any one of SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253 or SEQ ID NO: 254;

(B14) a protein comprising the amino acid sequence shown in SEQ ID NO: 245;

(B15) a protein comprising the amino acid sequence shown in SEQ ID NO: 247;

(B16) a protein comprising the amino acid sequence shown in SEQ ID NO: 248;

(B17) a protein comprising the amino acid sequence shown in SEQ ID NO: 249;

(B18) a protein comprising the amino acid sequence shown in SEQ ID NO: 250;

(B19) a protein comprising the amino acid sequence shown in SEQ ID NO: 251;

(B20) a protein comprising the amino acid sequence shown in SEQ ID NO: 252;

(B21) a protein comprising the amino acid sequence shown in SEQ ID NO: 253; and

(B22) a protein comprising the amino acid sequence shown in SEQ ID NO: 254;

32. A DNA comprising a nucleotide sequence selected from the group consisting of:

(ab1) a nucleotide sequence shown in SEQ ID NO: 9;

(ab2) a nucleotide sequence shown in SEQ ID NO: 10;

(ab3) a nucleotide sequence shown in SEQ ID NO: 11;

(ab4) a nucleotide sequence shown in SEQ ID NO: 110;

(ab5) a nucleotide sequence shown in SEQ ID NO: 144;

(ab6) a nucleotide sequence shown in SEQ ID NO: 145;

(ab7) a nucleotide sequence shown in SEQ ID NO: 146;

(ab8) a nucleotide sequence shown in SEQ ID NO: 147;

(ab9) a nucleotide sequence shown in SEQ ID NO: 148;

(ab10) a nucleotide sequence shown in SEQ ID NO: 235;

(ab11) a nucleotide sequence shown in SEQ ID NO: 236;

(ab12) a nucleotide sequence shown in SEQ ID NO: 237;

(ab13) a nucleotide sequence shown in SEQ ID NO: 238;

(ab14) a nucleotide sequence shown in SEQ ID NO: 239;

(ab15) a nucleotide sequence shown in SEQ ID NO: 240;

(ab16) a nucleotide sequence shown in SEQ ID NO: 241;

(ab17) a nucleotide sequence shown in SEQ ID NO: 242;

(ab18) a nucleotide sequence shown in SEQ ID NO: 243; and

(ab19) a nucleotide sequence shown in SEQ ID NO: 244;

33. A vector comprising the DNA according to the above 32;

34. A transformant in which the DNA according to the above 32 is introduced into a host cell;

35. The transformant according to the above 34, wherein the host cell is a microorganism cell or a plant cell;

36. A method of producing a transformant comprising a step of introducing into a host cell the DNA according to the above 32;

37. A method of producing a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method comprising a step of culturing the transformant according to the above 34 and recovering the produced said protein;

38. A method of controlling weeds comprising a step of applying a compound to a cultivation area of a plant expressing at least one herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO; 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

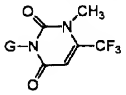
(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport

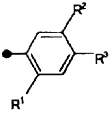
system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224,

wherein said compound is a compound of formula (I):

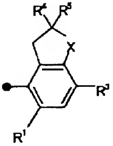


(I)

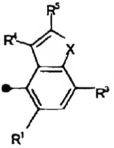
wherein in formula (I) G represents a group shown in any one of the following G-1 to G-9:



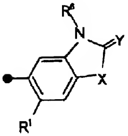
G-1



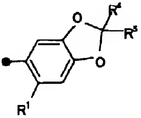
G-2



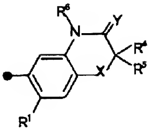
G-3



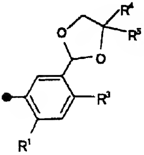
G-4



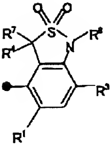
G-5



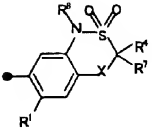
G-6



G-7



G-8



G-9

wherein in G-1 to G-9,

X represents an oxygen atom or sulfur atom;

Y represents an oxygen atom or sulfur atom;

R^1 represents a hydrogen atom or halogen atom;

R^2 represents a hydrogen atom, C_1-C_8 alkyl group, C_1-C_8 haloalkyl group, halogen atom, hydroxyl group, -OR^9 group, -SH group, -$S(O)pR^9$ group, -COR^9 group, -CO_2R^9 group, -$C(O)SR^9$ group, -$C(O)NR^{11}R^{12}$ group, -$CONH_2$ group, -CHO group, -CR^9=NOR^{18} group, -CH=$CR^{19}CO_2R^9$ group, -$CH_2CHR^{19}CO_2R^9$ group, -CO_2N=$CR^{13}R^{14}$ group, nitro group, cyano group, -$NHSO_2R^{15}$ group, -$NHSO_2NHR^{15}$ group, -NR^9R^{20} group, -NH_2 group or phenyl group that may be substituted with one or more C_1-C_4 alkyl groups which may be the same or different;

p represents 0, 1 or 2;

R^3 represents C_1-C_2 alkyl group, C_1-C_2 haloalkyl group, -OCH_3 group, -SCH_3 group, -$OCHF_2$ group, halogen atom, cyano group, nitro group or C_1-C_3 alkoxy group substituted with a phenyl group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_3 alkyl group, C_1-C_3 haloalkyl group, OR^{28} group, $NR^{11}R^{28}$ group, SR^{28} group, cyano group, CO_2R^{28} group and nitro group;

R^4 represents a hydrogen atom, C_1-C_3 alkyl group or C_1-C_3 haloalkyl group;

R^5 represents a hydrogen atom, C_1-C_3 alkyl group, C_1-C_3 haloalkyl group, cyclopropyl group, vinyl group, C_2 alkynyl group, cyano group, -$C(O)R^{20}$ group, -CO_2R^{20} group, -$C(O)NR^{20}R^{21}$ group, -$CHR^{16}R^{17}CN$ group, -$CR^{16}R^{17}C(O)R^{20}$ group, -$C^{16}R^{17}CO_2R^{20}$ group, -$CR^{16}R^{17}C(O)NR^{20}R^{21}$ group, -$CHR^{16}OH$ group, -$CHR^{16}OC(O)R^{20}$ group or -$OCHR^{16}OC(O)NR^{20}R^{21}$ group, or, when G represents G-2 or G-6, R^4 and R^5 may represent C=O group together with the carbon atom to which they are attached;

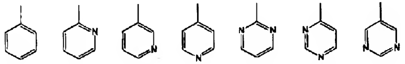
R^6 represents C_1-C_6 alkyl group, C_1-C_6 haloalkyl group, C_2-C_6 alkoxyalkyl group, C_3-C_6alkenyl group or C_3-C_6 alkynyl group;

R^7 represents a hydrogen atom, C_1-C_6 alkyl group, C_1-C_6 haloalkyl group, halogen atom, -$S(O)_2(C_1$-C_6 alkyl) group or -$C(=O)R^{22}$ group;

R^8 represents a hydrogen atom, C_1-C_8 alkyl group, C_3-C_8 cycloalkyl group, C_3-C_8 alkenyl group, C_3-C_8 alkynyl group, C_1-C_8 haloalkyl group, C_2-C_8 alkoxyalkyl group, C_3-C_8 alkoxyalkoxyalkyl group, C_3-C_8 haloalkynyl group, C_3-C_8 haloalkenyl group, C_1-C_8 alkylsulfonyl group, C_1-C_8 haloalkylsulfonyl group, C_3-C_8 alkoxycarbonylalkyl group, -$S(O)_2NH(C_1$-C_8 alkyl) group, -$C(O)R^{23}$ group or benzyl group which may be substituted with R^{24} on the phenyl ring;

R^9 represents C_1-C_8 alkyl group, C_3-C_8 cycloalkyl group, C_3-C_8 alkenyl group, C_3-C_8 alkynyl group, C_1-C_8 haloalkyl group, C_2-C_8 alkoxyalkyl group, C_2-C_8 alkylthioalkyl group, C_2-C_8 alkylsulfinylalkyl group, C_2-C_8 alkylsulfonylalkyl group, C_4-C_8 alkoxyalkoxyalkyl group, C_4-C_8 cycloalkylalkyl group, C_4-C_8 cycloalkoxyalkyl group, C_4-C_8 alkenyloxyalkyl group, C_4-C_8 alkynyloxyalkyl group, C_3-C_8 haloalkoxyalkyl group, C_4-C_8 haloalkenyloxyalkyl group, C_4-C_8 haloalkynyloxyalkyl group, C_4-C_8 cycloalkylthioalkyl group, C_4-C_8 alkenylthioalkyl group, C_4-C_8 alkynylthioalkyl group, C_1-C_4 alkyl group substituted with a phenoxy group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_3 alkyl group and C_1-C_3 haloalkyl group, C_1-C_4 alkyl group substituted with a benzyloxy group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_3 alkyl group and C_1-C_3 haloalkyl group, C_4-C_8 trialkylsyrylalkyl group, C_2-C_8 cyanoalkyl group, C_3-C_8 halocycloalkyl group, C_3-C_8 haloalkenyl group, C_5-C_8 alkoxyalkenyl group, C_5-C_8 haloalkoxyalkenyl group, C_5-C_8 alkylthioalkenyl group, C_3-C_8 haloalkynyl group, C_5-C_8 alkoxyalkynyl group, C_5-C_8 haloalkoxyalkynyl group, C_5-C_8 alkylthioalkynyl group, C_2-C_8 alkylcarbonyl group, benzyl group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_3 alkyl group, C_1-C_3 haloalkyl group, -OR^{28} group, -$NR^{11}R^{28}$ group, -SR^{28} group, cyano group, -CO_2R^{28} group and nitro group, -$CR^{16}R^{17}COR^{10}$ group, -$CR^{16}R^{17}CO_2R^{20}$ group,

$-CR^{16}R^{17}P(O)(OR^{10})_2$ group, $-CR^{16}R^{17}P(S)(OR^{10})_2$ group, $-CR^{16}R^{17}C(O)NR^{11}R^{12}$ group, $-CR^{16}R^{17}C(O)NH_2$ group, $-C(=CR^{26}R^{27})COR^{10}$ group, $-C(=CR^{26}R^{27})CO_2R^{20}$ group, $-C(=CR^{26}R^{27})P(O)(OR^{10})_2$ group, $-C(=CR^{26}R^{27})P(S)(OR^{10})_2$ group, $-C(=CR^{26}R^{27})C(O)NR^{11}R^{12}$ group, $-C(=CR^{26}R^{27})C(O)NH_2$ group, or any one of rings shown in Q-1 to Q-7:



Q-1 Q-2 Q-3 Q-4 Q-5 Q-6 Q-7

which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_6 alkyl group, C_1-C_6 haloalkyl group, C_2-C_6 alkenyl group, C_2-C_6 haloalkenyl group, C_2-C_6 alkynyl group, C_3-C_6 haloalkynyl group, C_2-C_8 alkoxyalkyl group, $-OR^{28}$ group, $-SR^{28}$ group, $-NR^{11}R^{28}$ group, C_3-C_8 alkoxycarbonylalkyl group, C_2-C_4 carboxyalkyl group, $-CO_2R^{28}$ group and cyano group;

R^{10} represents a C_1-C_6 alkyl group, C_2-C_6 alkenyl group, C_3-C_6 alkynyl group or tetrahydrofuranyl group;

R^{11} and R^{13} independently represent a hydrogen atom or C_1-C_4 alkyl group;

R^{12} represents C_1-C_6 alkyl group, C_3-C_6 cycloalkyl group, C_3-C_6 alkenyl group, C_3-C_6 alkynyl group, C_2-C_6 alkoxyalkyl group, C_1-C_6 haloalkyl group, C_3-C_6 haloalkenyl group, C_3-C_6 haloalkynyl group, phenyl group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_4 alkyl group and C_1-C_4 alkoxy group or $-CR^{16}R^{17}CO_2R^{25}$ group; or,

R^{11} and R^{12} together may represent $-(CH_2)_5-$, $-(CH_2)_4-$ or $-CH_2CH_2OCH_2CH_2-$, or in that case the resulting ring may be substituted with a substituent selected from a C_1-C_3 alkyl group, a phenyl group and benzyl group;

R^{14} represents a C_1-C_4 alkyl group or phenyl group which may be substituted on

the ring with a substituent selected from a halogen atom, C_1-C_3 alkyl group and C_1-C_3 haloalkyl group; or,

R^{13} and R^{14} may represent C_3-C_8 cycloalkyl group together with the carbon atom to which they are attached;

R^{15} represents C_1-C_4 alkyl group, C_1-C_4 haloalkyl group or C_3-C_6 alkenyl group;

R^{16} and R^{17} independently represent a hydrogen atom or C_1-C_4 alkyl group, C_1-C_4 haloalkyl group, C_2-C_4 alkenyl group, C_2-C_4 haloalkenyl group, C_2-C_4 alkynyl group, C_3-C_4 haloalkynyl group; or,

R^{16} and R^{17} may represent C_3-C_6 cycloalkyl group with the carbon atom to which they are attached, or the ring thus formed may be substituted with at least one substituent selected from a halogen atom, a C_1-C_3 alkyl group and C_1-C_3 haloalkyl group;

R^{18} represents a hydrogen atom, C_1-C_6 alkyl group, C_3-C_6 alkenyl group or C_3-C_6 alkynyl group;

R^{19} represents a hydrogen atom, C_1-C_4 alkyl group or halogen atom,

R^{20} represents a hydrogen atom, C_1-C_6 alkyl group, C_3-C_6 cycloalkyl group, C_3-C_6 alkenyl group, C_3-C_6 alkynyl group, C_2-C_6 alkoxyalkyl group, C_1-C_6 haloalkyl group, C_3-C_6 haloalkenyl group, C_3-C_6 haloalkynyl group, phenyl group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_4 alkyl group and $-OR^{28}$ group, or $-CR^{16}R^{17}CO_2R^{25}$ group;

R^{21} represents a hydrogen atom, C_1-C_2 alkyl group or $-CO_2(C_1$-C_4 alkyl) group;

R^{22} represents a hydrogen atom, C_1-C_6 alkyl group, C_1-C_6 alkoxy group or $NH(C_1$-C_6 alkyl) group;

R^{23} represents C_1-C_6 alkyl group, C_1-C_6 haloalkyl group, C_1-C_6 alkoxy group, $NH(C_1$-C_6 alkyl) group, benzyl group, C_2-C_8 dialkylamino group or phenyl group which may be substituted with R^{24};

R^{24} represents C_1-C_6 alkyl group, 1 to 2 halogen atoms, C_1-C_6 alkoxy group or CF_3 group;

R^{25} represents C_1-C_6 alkyl group, C_1-C_6 haloalkyl group, C_3-C_6 alkenyl group, C_3-C_6 haloalkenyl group, C_3-C_6 alkynyl group or C_3-C_6 haloalkynyl group;

R^{26} and R^{27} each represent independently a hydrogen atom, C_1-C_4 alkyl group, C_1-C_4 haloalkyl group, C_2-C_4 alkenyl group, C_2-C_4 haloalkenyl group, C_2-C_4 alkynyl group, C_3-C_4 haloalkynyl group, -OR^{28} group, -NHR^{28} group, or -SR^{28} group; or,

R^{26} and R^{27} may represent C_3-C_8 cycloalkyl group with the carbon atom to which they are attached, or each of the ring thus formed may be substituted with at least one substituent selected from a halogen atom, C_1-C_3 alkyl group and C_1-C_3 haloalkyl group; and,

R^{28} represents a hydrogen atom, C_1-C_6 alkyl group, C_1-C_6 haloalkyl group, C_3-C_6 alkenyl group, C_3-C_6 haloalkenyl group, C_3-C_6 alkynyl group, C_3-C_6 haloalkynyl group, C_2-C_4 carboxyalkyl group, C_3-C_8 alkoxycarbonylalkyl group, C_3-C_8 haloalkoxycarbonylalkyl group, C_5-C_9 alkenyloxycabonylalkyl group, C_5-C_9 haloalkenyloxycabonylalkyl group, C_5-C_9 alkynyloxycabonylalkyl group, C_5-C_9 haloalkynyloxycabonylalkyl group, C_5-C_9 cycloalkoxycabonylalkyl group or C_5-C_9 halocycloalkoxycabonylalkyl group;

39. A method of controlling weeds comprising a step of applying a compound to a cultivation area of a plant expressing at least one protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224;

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising the nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal

DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis, Streptomyces steffisburgensis or Saccharopolyspora taberi;

40. A method of evaluating the resistance of a cell to a compound of formula (I), said method comprising:

(1) a step of contacting said compound with a cell expressing at least one herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID

NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(2) a step of evaluating the degree of damage to the cell which contacted the compound in the above step (1);

41. The method according to the above 40, wherein the cell is a microorganism cell or plaint cell;

42. A method of selecting a cell resistant to a compound of formula (I), said method comprising a step of selecting a cell based on the resistance evaluated in the method according to the above 40;

43. The cell resistant to herbicide selected by the method according to the above 42, or the culture thereof;

44. A method of evaluating the resistance of a plant to a compound of formula (I), said method comprising:

(1) a step of contacting said compound with a plant expressing at least one herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequences shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID

NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(2) a step of evaluating the degree of damage to the plant which contacted the compound described in step (1);

45. A method of selecting a plant resistant to a compound of formula (I), said method comprising a step of selecting a plant based on the resistance evaluated in the method according to the above 44;

46. A herbicidally resistant plant selected from the method according to the above 45, or the progeny thereof;

47. A method of treating a compound of formula (I), said method comprising reacting said compound in the presence of an electron transport system containing an electron donor, with at least one herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport

system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224;

48. the method according to the above 47, wherein reacting the compound with the herbicide metabolizing protein by contacting the compound with a transformant in which a DNA encoding the herbicide metabolizing protein is introduced into a host cell in a position enabling its expression in said cell;

49. Use for treating the compound of formula (I) of a herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding any one of the amino acid sequences shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of

formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ

ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding the amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

50. Use for treating a compound of formula (I) of a polynucleotide encoding a herbicide metabolizing protein selected from the group consisting of

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein comprising an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215,

SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows the annealing site of the PCR primers utilized to obtain the present invention DNA (A1) and the present invention DNA (B1). Each of the numbers refers to the SEQ ID number showing the nucleotide sequence of the primers. The arrows show the annealing sites of the oligonucleotide primers having the nucleotide sequence shown with the SEQ ID number thereof and the extention direction of the DNA polymerase reaction from the primers. The dotted lines represent the DNA amplified by the PCR utilizing the primers. The thick line represents the region adjacent to the DNA insertion site of the vector utilized to produce the chromosomal DNA library.

Fig. 2 shows the annealing site of the PCR primers utilized to obtain the present invention DNA (A2) and the present invention DNA (B2). Each of the numbers refers to the SEQ ID number showing the nucleotide sequence of the primers. The arrows show the annealing sites of the oligonucleotide primers having the nucleotide sequence shown with the SEQ ID number thereof and the extention direction of the DNA polymerase reaction from the primers. The dotted lines represent the DNA amplified by the PCR utilizing the primers. The thick line represents the region adjacent to the DNA insertion site of the vector utilized to produce the chromosomal DNA library.

Fig. 3 shows the annealing site of the PCR primers utilized to obtain the present invention DNA (A4) and the present invention DNA (B4). Each of the numbers refers to the SEQ ID number showing the nucleotide sequence of the primers. The arrows show the annealing sites of the oligonucleotide primers having the nucleotide sequence shown

with the SEQ ID number thereof and the extention direction of the DNA polymerase reaction from the primers. The dotted lines represent the DNA amplified by the PCR utilizing the primers. The thick line represents the region adjacent to the DNA insertion site of the vector utilized to produce the chromosomal DNA library. However, the oligonucleotide primer represented by 57, is a primer which anneals to the region adjacent to the DNA insertion site of the vector utilized to produce the chromosomal DNA library, and fails to anneal with the present invention DNA (A4).

Fig. 4 shows the restriction map of the plasmid pKSN2.

Fig. 5 shows the restriction map of the plasmid pCRrSt12.

Fig. 6 shows the restriction map of the plasmid pCR657ET.

Fig. 7 shows the restriction map of the plasmid pCR657FET.

Fig. 8 shows the restriction map of the plasmid pCR657Bs.

Fig. 9 shows the restriction map of the plasmid pCR657FBs.

Fig. 10 shows the restriction map of the plasmid pUCrSt12.

Fig. 11 shows the restriction map of the plasmid pUCrSt657.

Fig. 12 shows the restriction map of the plasmid pUCrSt657F.

Fig. 13 shows the restriction map of the plasmid pUCCR16G6-p/t.

Fig. 14 shows the structure of the linker NotI-EcoRI produced by annealing the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 89 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 90.

Fig. 15 shows the restriction map of the plasmid pUCCR16G6-p/t Δ.

Fig. 16 shows the structure of the linker HindIII-NotI produced by annealing the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 91 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 92.

Fig. 17 shows the restriction map of the plasmid pNdG6- Δ T.

Fig. 18 shows the restriction map of the plasmid pSUM-NdG6-rSt657.

Fig. 19 shows the restriction map of the plasmid pSUM-NdG6-rSt657F.

Fig. 20 shows the restriction map of the plasmid pKFrSt12.

Fig. 21 shows the restriction map of the plasmid pKFrSt12-657.

Fig. 22 shows the restriction map of the plasmid pKFrSt12-657F.

Fig. 23 shows the restriction map of the plasmid pSUM-NdG6-rSt12-657.

Fig. 24 shows the restriction map of the plasmid pSUM-NdG6-rSt12-657F.

Fig. 25 shows the structure of the linker HindIII-NotI-EcoRI produced by annealing the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 98 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 99.

Fig. 26 shows the restriction map of the plasmid pBI121S.

Fig. 27 shows the restriction map of the plasmid pBI-NdG6-rSt-657.

Fig. 28 shows the restriction map of the plasmid pBI-NdG6-rSt-657F.

Fig. 29 shows the restriction map of the plasmid pBI-NdG6-rSt12-657.

Fig. 30 shows the restriction map of the plasmid pBI-NdG6-rSt12-657F.

Fig. 31 shows the restriction map of the plasmid pCR923Sp.

Fig. 32 shows the restriction map of the plasmid pNdG6-rSt12.

Fig. 33 shows the restriction map of the plasmid pSUM-NdG6-rSt-923.

Fig. 34 shows the restriction map of the plasmid pKFrSt12-923.

Fig. 35 shows the restriction map of the plasmid pSUM-NdG6-rSt12-923.

Fig. 36 shows the restriction map of the plasmid pBI-NdG6-rSt-923.

Fig. 37 shows the restriction map of the plasmid pBI-NdG6-rSt12-923.

Fig. 38 shows the restriction map of the plasmid pCR671ET.

Fig. 39 shows the restriction map of the plasmid pCR671Bs.

Fig. 40 shows the restriction map of the plasmid pUCrSt671.

Fig. 41 shows the restriction map of the plasmid pSUM-NdG6-rSt-671.

Fig. 42 shows the restriction map of the plasmid pKFrSt12-671.

Fig. 43 shows the restriction map of the plasmid pSUM-NdG6-rSt12-671.

Fig. 44 shows the restriction map of the plasmid pBI-NdG6-rSt-671.

Fig. 45 shows the restriction map of the plasmid pBI-NdG6-rSt12-671.

Fig. 46 shows the results obtained by detecting with agarose gel electrophoresis the DNA amplified by the PCR using as a primer the oligonucleotide having a partial nucleotide sequence of the present invention DNA(A). Lanes 1, 7, 8, 12, 19, 26, 27, 32, 37, 42 and 47 represent the electrophoresis of a DNA marker (ϕ 174/HaeIII digest). The other lanes represent the electrophoresis of the samples shown in Tables 20 and 21.

Fig. 47 shows the structure of the linker produced by annealing the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 134 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 135.

Fig. 48 shows the restriction map of the plasmid pUCrSt657soy.

Fig. 49 shows the restriction map of the plasmid pSUM-NdG6-rSt-657soy.

Fig. 50 shows the restriction map of the plasmid pKFrSt12-657soy.

Fig. 51 shows the restriction map of the plasmid pSUM-NdG6-rSt12-657soy.

Fig. 52 shows the restriction map of the plasmid pBI-NdG6-rSt-657soy.

Fig. 53 shows the restriction map of the plasmid pBI-NdG6-rSt12-657soy.

Fig. 54 shows the restriction map of the plasmid pUCrSt1584soy.

Fig. 55 shows the restriction map of the plasmid pSUM-NdG6-rSt-1584soy.

Fig. 56 shows the restriction map of the plasmid pKFrSt12-1584soy.

Fig. 57 shows the restriction map of the plasmid pSUM-NdG6-rSt12-1584soy.

Fig. 58 shows the restriction map of the plasmid pBI-NdG6-rSt-1584soy.

Fig. 59 shows the restriction map of the plasmid pBI-NdG6-rSt12-1584soy.

Fig. 60 shows the restriction map of the plasmid pUCrSt1609soy.

Fig. 61 shows the restriction map of the plasmid pSUM-NdG6-rSt-1609soy.

Fig. 62 shows the structure of the linker EcoT22I-12aa-EcoT22I produced by annealing the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 402 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 403.

Fig. 63 shows the restriction map of the plasmid pUCrSt12-1609soy.

Fig. 64 shows the restriction map of the plasmid pSUM-NdG6-rSt12-1609soy.

Fig. 65 shows the restriction map of the plasmid pBI-NdG6-rSt-1609soy.

Fig. 66 shows the restriction map of the plasmid pBI-NdG6-rSt12-1609soy.

The abbreviations described in the above figures are explained below.

DNA A1: the present invention DNA (A1)

DNA A2: the present invention DNA (A2)

DNA A3: the present invention DNA (A3)

DNA A4: the present invention DNA (A4)

DNA B1: the present invention DNA (B1)

DNA B2: the present invention DNA (B2)

DNA B4: the present invention DNA (B4)

DNA A1S: the present invention DNA (A1)S

DNA A23S: the present invention DNA (A23)S

DNA A25S: the present invention DNA (A25)S

tac p: tac promoter

rrnB t: rrnB terminator

ColE1 ori: the replication origin of plasmid ColE1

Amp^r: the ampicillin resistance gene

RuBPCssCTP: the nucleotide sequence encoding the chloroplast transit peptide of the small subunit of ribulose-1,5-bisphosphate carboxylase of soybean (cv. Jack).

12aa: the nucleotide sequence encoding the 12 amino acids of a mature protein, following the chloroplast transit peptide of the small subunit of ribulose-1,5-bisphosphate carboxylase of soybean (cv. Jack).

Km^r: kanamycin resistance gene

F1 ori: replication origin of plasmid F1

CR16G6p: CR16G6 promoter

CR16t: CR16 terminator

CR16tΔ: DNA in which the nucleotide sequence downstream of restriction site of the restriction enzyme ScaI is removed from the CR16 terminator

CR16G6pΔ: DNA in which the nucleotide sequence upstream of restriction site of the restriction enzyme NdeI is removed from the CR16G6 terminator

NOSp: promoter of the nopaline synthase gene

NPTII: kanamycin resistance gene

NOSt: terminator of nopaline synthase gene

GUS: β-glucuronidase gene

RB: the right border sequence of T-DNA

LB: the left border sequence of T-DNA

NdeI, HindIII, BspHI, EcoRI, BamHI, EcoT22I, SphI, KpnI, SacI, BglII, NotI, ScaI: the

restriction sites of the respective restriction enzyme

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention is explained in detail below.

The herbicide metabolizing protein selected from the following protein group (hereinafter, sometimes referred to as "the present invention protein (A)") has the ability to convert the compound of formula (II) (hereinafter, sometimes referred to as "compound (II)") to the compound of formula (III) (hereinafter, sometimes referred to as "compound (III)").

<protein group>

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224;

(A27) a protein having the ability to convert in the presence of an electron transport

system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising the nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis, Streptomyces steffisburgensis or Saccharopolyspora taberi.

As specific examples of the present invention protein (A), there is mentioned:

a protein comprising the amino acid sequence shown in SEQ ID NO: 1 (hereinafter, sometimes referred to as "present invention protein (A1)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 2 (hereinafter, sometimes referred to as "present invention protein (A2)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 3 (hereinafter, sometimes referred to as "present invention protein (A3)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 108 (hereinafter, sometimes referred to as "present invention protein (A4)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 159 (hereinafter, sometimes referred to as "present invention protein (A11)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 160 (hereinafter, sometimes referred to as "present invention protein (A12)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 136 (hereinafter, sometimes referred to as "present invention protein (A13)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 137 (hereinafter, sometimes referred to as "present invention protein (A14)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 138 (hereinafter, sometimes referred to as "present invention protein (A15)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 215 (hereinafter, sometimes referred to as "present invention protein (A16)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 216 (hereinafter, sometimes referred to as "present invention protein (A17)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 217 (hereinafter, sometimes referred to as "present invention protein (A18)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 218 (hereinafter, sometimes referred to as "present invention protein (A19)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 219

(hereinafter, sometimes referred to as "present invention protein (A20)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 220 (hereinafter, sometimes referred to as "present invention protein (A21)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 221 (hereinafter, sometimes referred to as "present invention protein (A22)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 222 (hereinafter, sometimes referred to as "present invention protein (A23)");

a protein comprising the amino acid sequence shown in SEQ ID NO: 223 (hereinafter, sometimes referred to as "present invention protein (A24)"); and

a protein comprising the amino acid sequence shown in SEQ ID NO: 224 (hereinafter, sometimes referred to as "present invention protein (A25)").

For example, by reacting the PPO inhibitory-type herbicidal compound of formula (I) (hereinafter, sometimes referred to as "compound (I)") with the present invention protein (A), it is capable to convert the compound to a compound with lower herbicidal activity.

Further, in treatment to convert compound (I) to a compound of a lower herbicidal activity, there can also be utilized a herbicide metabolizing protein selected from the following group (hereinafter, sometimes referred to as "present protein (A)"):

<protein group>

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system

containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of

formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224.

As examples of the present protein (A), there can be mentioned the present invention protein A, described above. Further, as other examples, there can be mentioned

a protein comprising the amino acid sequence shown in SEQ ID NO: 4 (hereinafter, sometimes referred to as "present protein (A9)") and

a protein comprising the amino acid sequence shown in SEQ ID NO: 5 (hereinafter, sometimes referred to as "present protein (A10)").

In the amino acid sequence of the protein shown in (A5), (A6), (A7), (A8), (A26), (A27) or (A28) in the above protein groups, the differences which may be observed from the amino acid sequences shown in SEQ ID NO: 1, 2, 3, 108, 159, 160, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224, are such as deletion, substitution, and addition of certain amino acids. Such differences include, for example, the deletion from the processing which the above protein comprising the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 108, 159, 160, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224 receives within the cell. Further, there are included a polymorphic variation which occurs naturally resulting from the difference by such as the species, individual or the like of the organism from which the protein is derived; amino acid deletions,

substitutions, and additions arising from genetic mutations artificially introduced by such as a site-directed mutagenesis method, a random mutagenesis method, a mutagenic treatment and the like.

The number of amino acids undergoing such deletions, substitutions and additions may be within the range in which the present protein (A) can develop the ability to convert compound (II) to compound (III). Further, as a substitution of the amino acid, there can be mentioned, for example, substitutions to an amino acid which is similar in hydrophobicity, charge, pK, stereo-structural feature, or the like. As such substitutions, specifically for example, there are mentioned substitutions within the groups of: (1.) glycine and alanine; (2.) valine, isoleucine and leucine; (3.) aspartic acid, glutamic acid, asparagine and glutamine; (4.) serine and threonine; (5.) lysine and arginine; (6.) phenylalanine and tyrosine; and the like.

Further, in the present protein (A), it is preferable that the cysteine present at the position aligning to the cysteine of amino acid number 357 in the amino acid sequence shown in SEQ ID NO: 1 is conserved (not undergo a deletion or substitution): examples of such cysteine include the cysteine shown at amino acid number 350 in the amino acid sequence shown in SEQ ID NO: 2, the cysteine shown at amino acid number 344 in the amino acid sequence shown in SEQ ID NO: 3, the cysteine shown at amino acid number 360 in the amino acid sequence shown in SEQ ID NO: 108; the cysteine shown at amino acid number 359 in the amino acid sequence shown in SEQ ID NO: 4, the cysteine shown at amino acid number 355 in the amino acid sequence shown in SEQ ID NO: 5, the cysteine shown at amino acid number 358 in the amino acid sequence shown in SEQ ID NO: 159, the cysteine shown at amino acid number 374 in the amino acid sequence shown in SEQ ID NO: 160, the cysteine shown at amino acid number 351 in the amino acid sequence shown in SEQ ID NO: 136, the cysteine shown at amino acid number 358

in the amino acid sequence shown in SEQ ID NO: 137, the cysteine shown at amino acid number 358 in the amino acid sequence shown in SEQ ID NO: 138, the cysteine shown at amino acid number 347 in the amino acid sequence shown in SEQ ID NO: 222, the cysteine shown at amino acid number 347 in the amino acid sequence shown in SEQ ID NO: 224 and the like.

As methods of artificially causing such amino acid deletions, additions or substitutions (hereinafter, sometimes, collectively referred to as "amino acid modification"), for example, there is mentioned a method comprising the steps of carrying out site-directed mutagenesis on the DNA encoding an amino acid sequence shown in any one of SEQ ID NO: 1, 2, 3, 108, 159, 160, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224, and then allowing the expression of such DNA by a conventional method. As the site-directed mutagenesis method, for example, there is mentioned a method which utilizes amber mutations (Gapped Duplex method, Nucleic Acids Res., 12, 9441-9456 (1984)), a method by PCR utilizing primers for introducing a mutation and the like. Further, as methods of artificially modifying amino acids, for example, there is mentioned a method comprising the steps of carrying out random mutagenesis on the DNA encoding any one of the amino acid sequences shown in SEQ ID NO: 1, 2, 3, 108, 159, 160, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224 and then allowing the expression of such DNA by a conventional method. As the random mutagenesis method, for example, there is mentioned method of conducting PCR by utilizing the DNA encoding any one of the above amino acid sequences as a template an by utilizing a primer pair which can amplify the full length of each of the DNA, under the condition in which the concentration of each of dATP, dTTP, dGTP and dCTP, utilized as a substrate, are different than usual or under the condition in which the concentration of Mg^{2+} that promotes the polymerase reaction is increased to more than

usual. As such methods of PCR, for example, there is mentioned the method described in Method in Molecular Biology, (31), 1994, 97-112. Further, there may be mentioned the method described in PCT patent publication WO 00/09682.

In the present invention, "sequence identity" refers to the homology and identity between two nucleotide sequences or two amino acid sequences. Such "sequence identity" may be determined by comparing the two sequences, each aligned in an optimal state, over the whole region of the test sequences. As such, additions or deletions (for example, gaps) can be utilized in the optimal alignment of the test nucleic acid sequences or amino acid sequences. Such sequence identity can be calculated through the step of producing the alignment conducted by a homology analysis using a program such as FASTA (Pearson & Lipman, Proc. Natl. Acad. Sci. USA, 4, 2444-2448 (1988)), BLAST (Altschul et al., Journal of Molecular Biology, 215, 403-410 (1990)), CLUSTAL W (Thompson, Higgins & Gibson, Nucleic Acid Research, 22, 4673-4680 (1994a)) and the like. Such programs, for example, can be typically utilized on the webpage (http://www.ddbj.nig.ac.jp) of the DNA Data Bank of Japan (the international databank operated within the Center for Information Biology and DNA Data Bank of Japan). Further, the sequence identity may be determined by utilizing a commercially available sequence analysis software. Specifically for example, it can be calculated by producing an alignment conducted by a homology analysis by the Lipman-Pearson method (Lipman, D.J. and Pearson, W.R., Science, 227, 1435-1441, (1985)) utilizing GENETYX-WIN Ver.5 (Software Development Company, Ltd.).

As the "stringent condition" described in (A7), there can be mentioned, for example, the conditions under which a hybrid is formed at 45℃ in a solution containing

6xSSC (let the solution containing 1.5 M NaCl and 0.15 M trisodium citrate be 10×SSC) and then the hybrid is washed at 50℃ with 2xSSC (Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6) in a hybridization conducted according to the conventional method described in such as Sambrook, J., Frisch, E.F., and Maniatis, T.; Molecular Cloning 2nd edition, Cold Spring Harbor Press. The salt concentration in the washing step can be selected, for example, from the conditions of 2 × SSC (low stringency condition) to the conditions of 0.2 × SSC (high stringency conditions). A temperature in the washing step can be selected, for example, from room temperature (low stringency condition) to 65°C (high stringency condition). Alternatively, both of the salt concentration and temperature may be changed.

As a DNA which "hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108", specifically for example, there can be mentioned a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, 2, 3, 4, 5, 108, 159, 160, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224, a DNA comprising a nucleotide sequence shown in any one of SEQ ID NO: 6, 7, 8, 78, 84, 109, 139, 140, 141, 142, 143, 225, 226, 227, 228, 229, 230, 231, 232, 233 or 234, and the like. There can also be mentioned DNA comprising a nucleotide sequence having at least about 60% identity to a nucleotide sequence shown in any one of SEQ ID NO: 6, 7, 8, 78, 84, 109, 139, 140, 141, 142, 143, 225, 226, 227, 228, 229, 230, 231, 232, 233 or 234.

The molecular weight of the present protein (A) is about 30,000 to 60,000 and is typically about 40,000 to 50,000 (comparable to, for example, a protein consisting of the

amino acid sequence shown in any one of SEQ ID NO: 1, 2, 3, 108, 159, 160, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224), as the molecular weight identified by a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (hereinafter, referred to as "SDS-PAGE"). Further, the present protein (A), as long as the ability to convert compound (II) to compound (II) is not eliminated, can be utilized as a protein to which amino acid sequence is added upstream to its amino terminus or downstream to its carboxy terminus.

As the marker of the abilityof the present protein (A) to metabolize the PPO inhibitory-type herbicidal compound of formula (I), there can be mentioned the ability to convert compound (II) to compound (III). Such ability, for example, can be confirmed by reacting compound (II) with the present protein (A) in the presence of an electron transport system containing an electron donor such as coenzyme NADPH and by detecting the produced compound (III).

The "electron transport system containing an electron donor" refers to a system in which a redox chain reaction occurs and an electron is transferred from the electron donor to the present protein (A). As the electron donor, for example, there is mentioned coenzymes NADPH, NADH and the like. For example, as proteins which may constitute the electron transport system from NADPH to the present protein (A), there is mentioned ferredoxin and ferredoxin-$NADP^+$ reductase, NADPH-cytochrome P-450 reductase, and the like.

To confirm the ability of converting compound (II) to compound (III), for example, a reaction solution of about pH 7, comprising the present protein (A), β-NADPH, ferredoxin, ferredoxin-$NADP^+$ reductase and compound (II) labeled with a radioisotope, is incubated at about 30℃ for about 10 minutes to 1 hour. Subsequently,

after making the reaction solution acidic by adding hydrochloric acid, it is extracted with ethyl acetate. After subjecting the recovered ethyl acetate layer to thin layered chromatography (hereinafter referred to as "TLC"), autoradiography is conducted and the ability to convert compound (II) to compound (III) can be confirmed by detecting the labeled compound (III).

To prepare the present protein (A), for example, first, the DNA encoding the present protein (A) (hereinafter, sometimes collectively referred to as "present DNA (A)") is obtained according to the conventional genetic engineering methods (for example, the methods described in Sambrook, J., Frisch, E.F., Maniatis, T.; Molecular Cloning 2nd Edition, Cold Spring Harbor Laboratory press).

As examples of the present DNA (A), there can be mentioned a DNA encoding the present invention protein (A) (hereinafter, sometimes referred to as "present invention DNA (A)"). As specific examples of the present invention DNA (A), there can be mentioned:

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 1 (hereinafter, sometimes referred to as "present invention DNA (A1)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 2 (hereinafter, sometimes referred to as "present invention DNA (A2)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 3 (hereinafter, sometimes referred to as "present invention DNA (A3)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 108 (hereinafter, sometimes referred to as "present invention DNA (A4)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 159 (hereinafter, sometimes referred to as "present invention DNA (A11)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 160 (hereinafter, sometimes referred to as "present invention DNA (A12)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 136 (hereinafter, sometimes referred to as "present invention DNA (A13)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 137 (hereinafter, sometimes referred to as "present invention DNA (A14)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 138 (hereinafter, sometimes referred to as "present invention DNA (A15)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 215 (hereinafter, sometimes referred to as "present invention DNA (A16)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 216 (hereinafter, sometimes referred to as "present invention DNA (A17)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 217 (hereinafter, sometimes referred to as "present invention DNA (A18)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 218 (hereinafter, sometimes referred to as "present invention DNA (A19)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 219 (hereinafter, sometimes referred to as "present invention DNA (A20)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 220 (hereinafter, sometimes referred to as "present invention DNA (A21)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 221 (hereinafter, sometimes referred to as "present invention DNA (A22)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 222 (hereinafter, sometimes referred to as "present invention DNA (A23)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID

NO: 223 (hereinafter, sometimes referred to as "present invention DNA (A24)");

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 224 (hereinafter, sometimes referred to as "present invention DNA (A25)"); and the like.

Further as more specific examples of the present invention DNA (A), there can be mentioned;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 6;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 9;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 7;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 10;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 8;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 11;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 109;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 110;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 139;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 144;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 140;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 145;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 141;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 146;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 142;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 147;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 143;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 148;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 225;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 235;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 226;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 236;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 227;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 237;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 228;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 238;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 229;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 239;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 230;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 240;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 231;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 241;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 232;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 242;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 233;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 243;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 234;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 244;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 214;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 368;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 393;

a DNA encoding a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and having at least 80% sequence identity with a nucleotide

sequence shown in any one of SEQ ID NO: 6, 7, 8 or 109;

a DNA encoding a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and having at least 90% sequence identity with a nucleotide sequences shown in any one of SEQ ID NO: 139, 140, 141, 142, 143, 225, 226, 227, 228, 229, 230, 231, 232, 233 or 234; and the like.

Further, as examples of the present DNA (A), other than the present invention DNA (A) above, there is mentioned:

a DNA comprising the nucleotide sequence encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 4 (hereinafter, sometimes referred to as "present DNA (A9)");

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 78;

a DNA comprising the nucleotide sequence encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 5 (hereinafter, sometimes referred to as "present DNA (A10)");

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 84;

a DNA comprising the nucleotide sequence shown in SEQ ID NO: 85; and the like.

The present DNA(A), for example, may be a DNA cloned from nature and may be a DNA in which a deletion, substitution or addition of nucleotide(s) has been introduced to the DNA cloned from nature by such as a site-directed mutagenesis method, a random mutagenesis method, and may be an artificially synthesized DNA. Subsequently, the present protein (A) can be produced or obtained by expressing the obtained present DNA (A) according to the conventional genetic engineering methods. In such ways, the present protein (A) can be prepared.

The present DNA (A) can be prepared, for example, by the following methods. First, chromosomal DNA is prepared by conventional genetic engineering methods, such as those described in Molecular Cloning: A Laboratory Manual 2nd edition (1989), Cold Spring Harbor Laboratory Press; and Current Protocols in Molecular Biology (1987), John Wiley & Sons, Incorporated, from microorganisms belonging to Streptomyces, such as Streptomyces phaeochromogenes, Streptomyces testaceus, Strepotomyces achromogenes, Streptomyces griseolus, Streptomyces carbophilus, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis and Streptomyces steffisburgensis, and more specifically, Streptomyces phaeochromogenes IFO12898, Streptomyces testaceus ATCC21469, Streptomyces achromogenes IFO 12735, Streptomyces griseolus ATCC11796, Streptomyces carbophilus SANK62585, Streptomyces griseofuscus IFO 12870t, Streptomyces thermocoerulescens IFO 14273t, Streptomyces nogalater IFO 13445, Streptomyces tsusimaensis IFO 13782, Streptomyces glomerochromogenes IFO 13673t, Streptomyces olivochromogenes IFO 12444, Streptomyces ornatus IFO 13069t, Streptomyces griseus ATCC 10137, Streptomyces griseus IFO 13849T, Streptomyces lanatus IFO 12787T, Streptomyces misawanensis IFO 13855T, Streptomyces pallidus IFO 13434T, Streptomyces roseorubens IFO 13682T, Streptomyces rutgersensis IFO 15875T and Streptomyces steffisburgensis IFO 13446T, and the like; or microorganisms belonging to Saccharopolyspora, such as Saccharopolyspora taberi, more specifically, Saccharopolyspora taberi JCM 9383t and the like. Next, after partial digestion of the

chromosomal DNA with a restriction enzyme such as Sau3AI, a DNA of about 2kb is recovered. The recovered DNA is cloned into a vector according to the conventional genetic engineering methods described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; and "Current Protocols in Molecular Biology" (1987), John Wiley & Sons, Incorporated. As the vector, specifically for example, there can be utilized pUC 119 (TaKaRa Shuzo Company), pTVA 118N (Takara Shuzo Company), pBluescript II (Toyobo Company), pCR2.1-TOPO (Invitrogen), pTrc99A (Amersham Pharmacia Biotech Company), pKK331-1A (Amersham Pharmacia Biotech Company), and the like. A chromosomal DNA library can be obtained by extracting the plasmid from the obtained clone.

The present DNA (A) can be obtained by hybridizing a probe with the obtained chromosomal DNA library under the conditions described below, and by detecting and recovering the DNA which bound specifically with the probe. The probe can be a DNA consisting of about at least 20 nucleotides comprising the nucleotides sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, 2, 3 or 108. As specific examples of the DNA which can be utilized as probes, there is mentioned a DNA comprising a nucleic acid shown in any one of SEQ ID NO: 6, 7, 8or 109; a DNA comprising a partial nucleotide sequence of the nucleic acid sequence shown in any one of SEQ ID NO: 6, 7, 8or 109; a DNA comprising a nucleotide sequence complimentary to said partial nucleotide sequence; and the like.

The DNA utilized as the probe is labeled with a radioisotope, fluorescent coloring or the like. To label the DNA with a radioisotope, for example, there can be utilized the Random Labeling Kit of Boehringer or Takara Shuzo Company. Further, a DNA labeled with ^{32}P can be prepared by conducting PCR. The DNA to be utilized for the probe is

utilized as the template. The dCTP typically utilized in the PCR reaction solution is exchanged with (α-^{32}P)dCTP. Further, when labeling the DNA with fluorescent coloring, for example, there can be utilized DIG-High Prime DNA labeling and Detection Starter Kit II (Roche Company).

A specific example of preparing the probe is explained next. For example, a DNA labeled with digoxigenin, comprising the full length of the nucleotide sequence shown in SEQ ID NO: 6 can be obtained by utilizing the chromosomal DNA prepared from Streptomyces phaeochromogenes IFO12898 as described above or a chromosomal DNA library as a template, by utilizing as primers an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 93 and an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 94, and by conducting PCR as described in the examples described below with, for example, PCR DIG Probe Synthesis Kit (Roche Diagnostics GmbH) according to the attached manual. Similarly, a DNA labeled with digoxigenin, comprising the nucleotide sequence of from nucleotide 57 to nucleotide 730 shown in SEQ ID NO: 6 can be obtained by utilizing the chromosomal DNA prepared from Streptomyces phaeochromogenes IFO12898 as described above or a chromosomal DNA library as the template. As primers, the PCR is conducted with an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 130 and an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 131. Further, a DNA labeled with digoxigenin, comprising the full length of the nucleotide sequence shown in SEQ ID NO: 7 can be obtained by utilizing the chromosomal DNA prepared from Saccharopolyspora taberi JCM 9383t as described above or a chromosomal DNA library as the template. As primers, the PCR is conducted with an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 61 and an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 62. Further, a DNA labeled with

digoxigenin, comprising the full length of the nucleotide sequence shown in SEQ ID NO: 8 can be obtained by utilizing the chromosomal DNA prepared from Streptomyces testaceus ATCC21469 as described above or a chromosomal DNA library as the template. As primers, the PCR is conducted with an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 70 and an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 71. Further, a DNA labeled with digoxigenin, comprising the nucleotide sequence of from nucleotide 21 to nucleotide 691 shown in SEQ ID NO: 8 can be obtained by utilizing the chromosomal DNA prepared from Streptomyces testaceus ATCC21469 as described above or a chromosomal DNA library as the template. As primers, the PCR is conducted with an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 132 and an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 133.

The methods by which a probe is allowed to hybridize with the chromosomal DNA library may include colony hybridization and plaque hybridization, and an appropriate method may be selected, which is compatible with the type of vector used in the library preparation. When the utilized library is constructed with the use of plasmid vectors, colony hybridization is conducted. Specifically first, transformants are obtained by introducing the DNA of the library into microorganism in which the plasmid vector utilized to construct the library is replicable. The obtained transformants are diluted and spread onto an agar plate and cultured until colonies appear. When a phage vector is utilized to construct the library, plaque hybridization is conducted. Specifically, first, the microorganism in which the phage vector utilized to produce the library is replicable is mixed with the phage of the library, under the conditions in which infection is possible. The mixture is then further mixed with soft agar. This mixture is then spread onto an

agar plate. Subsequently, the mixture is cultured until plaques appear.

Next, in the case of any one of the above hybridizations, a membrane is placed on the surface of the agar plate in which the above culturing was conducted and the colonies of the transformants or the phage particles in the plaques are transferred to the membrane. After alkali treatment of the membrane, there is a neutralization treatment. The DNA eluted from the transformants or the phage particles is then fixed onto the membrane. More specifically for example, in the event of plaque hybridization, the phage particles are absorbed onto the membrane by placing a nitrocellulose membrane or a nylon membrane, specifically for example, Hybond-N^+ (Amersham Pharmacia Biotech Company) on the agar plate and waiting for 1 minute. The membrane is soaked in an alkali solution (1.5M NaCl and 0.5N NaOH) for about 3 minutes to dissolve the phage particles and elute the phage DNA onto the membrane. The membrane is then soaked in neutralization solution (1.5M NaCl and 0.5M tris-HCl buffer pH7.5) for about 5 minutes. After washing the membrane in washing solution (0.3M NaCl, 30mM sodium citrate, 0.2M tris-HCl buffer pH7.5) for about 5 minutes, for example, the phage DNA is fixed onto the membrane by incubating about 80℃ for about 90 minutes in vacuo.

By utilizing the membrane prepared as such, hybridization is conducted with the above DNA as a probe. Hybridization can be conducted, for example, according to the description in "Molecular Cloning: A Laboratory Manual 2nd edition (1989)" Cold Spring Harbor Laboratory Press, and the like.

While various temperature conditions and reagents are available for conducting hybridization, the membrane prepared as described above is soaked with and maintained for 1 hour to 4 hours at 42℃ to 65℃ in a prehybridization solution, which is prepared at a ratio of from 50μl to 200μl per 1cm^2 of the membrane. The prehybridization solution, for example, may contain 450mM to 900mM NaCl and 45mM to 90mM sodium citrate,

contain sodium dodecyl sulfate (hereinafter, referred to as "SDS") at a concentration of 0.1% to 1.0%, and contain denatured unspecific DNA at a concentration of from 0μg/ml to 200μg/ml, and may sometimes contain albumin, phycol, and polyvinyl pyrrolidone, each at a concentration of 0% to 0.2%. Subsequently, for example, the membrane is soaked with and maintained for 12 hours to 20 hours at 42℃ to 65℃ in a hybridization solution, which is prepared at a ratio of from 50μl to 200μl per $1cm^2$ of the membrane. The hybridization solution is, for example, a mixture of the prehybridization solution, which may contain 450mM to 900mM NaCl and 45mM to 90mM sodium citrate, contain SDS at a concentration of 0.1% to 1.0%, and contain denatured unspecific DNA at a concentration of from 0μg/ml to 200μg/ml, and may sometimes contain albumin, phycol, and polyvinyl pyrrolidone, each at a concentration of 0% to 0.2%, with the probe obtained with the preparation method described above (in a relative amount of $1.0x10^4$ cpm to $2.0x10^6$ cpm per $1cm^2$ of the membrane). Subsequently, the membrane is removed and a wash of 5 minutes to 15 minutes is conducted about 2 to 4 times, utilizing a washing solution of 42℃ to 65℃ that contains 15mM to 300mM of NaCl, 1.5mM to 30mM of sodium citrate and 0.1% to 1.0% of SDS. Further, after lightly rinsing with 2xSSC solution (300mM NaCl and 30mM sodium citrate), the membrane is dried. By detecting the position of the probe on the membrane by subjecting the membrane to autoradiography, the position of the DNA hybridizing to the utilized probe on the membrane is identified. Alternatively, prehybridization and hybridization can be conducted with the use of a commercially available hybridization kit, such as with the use of hybridization solution contained in the DIG-High Prime DNA Labeling and Detection Starter Kit II (Roche). After hybridization, for example, the membrane is washed twice for 5 minutes at room temperature in 2xSSC containing 0.1% SDS, followed by washing twice for 15 minutes at 65℃ in 0.5xSSC containing 0.1% SDS. The positions of DNAs

on the membrane hybridizing with the utilized probe are detected, by treating in turn the washed membrane with the detection solution contained in the kit and by detecting the position of the probe on the membrane.

The clones corresponding to the positions of the detected DNAs on the membrane are identified on the original agar medium, and can be picked up to isolate clones carrying those DNAs.

The present DNA (A) obtained according to the above can be cloned into a vector according to conventional genetic engineering methods described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press, "Current Protocols in Molecular Biology" (1987), John Wiley & Sons Incorporated, and the like. As the vector, specifically for example, there can be utilized pUCA119 (Takara Shuzo Company), pTVA118N (Takara Shuzo Company), pBluescriptII (Toyobo Company), pCR2.1-TOPO (Invitrogen Company), pTrc99A (Pharmacia Company), pKK331-1A (Pharmacia Company) and the like.

Further, the nucleotide sequence of the present DNA (A) obtained according to the above description can be analyzed by the dideoxy terminator method described in F. Sanger, S. Nicklen, A.R. Coulson, Proceeding of National Academy of Science U.S.A. (1977) 74:5463-5467. In the sample preparation for the nucleotide sequence analysis, a commercially available reagent may be utilized, such as the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin Elmer Company.

The present DNA (A) can also be prepared as follows. The present DNA (A) can be amplified by conducting PCR. The PCR may utilize as a template the chromosomal DNA or chromosomal DNA library prepared as described above from microorganisms

belonging to Streptomyces, such as Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseolus, Streptomyces carbophilus, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis and Streptomyces steffisburgensis, and more specifically, Streptomyces phaeochromogenes IFO12898, Streptomyces testaceus ATCC21469, Streptomyces achromogenes IFO 12735, Streptomyces griseolus ATCC11796, Streptomyces carbophilus SANK62585, Streptomyces griseofuscus IFO 12870t, Streptomyces thermocoerulescens IFO 14273t, Streptomyces nogalater IFO 13445, Streptomyces tsusimaensis IFO 13782, Streptomyces glomerochromogenes IFO 13673t, Streptomyces olivochromogenes IFO 12444, Streptomyces ornatus IFO 13069t, Streptomyces griseus ATCC 10137, Streptomyces griseus IFO 13849T, Streptomyces lanatus IFO 12787T, Streptomyces misawanensis IFO 13855T, Streptomyces pallidus IFO 13434T, Streptomyces roseorubens IFO 13682T, Streptomyces rutgersensis IFO 15875T and Streptomyces steffisburgensis IFO 13446T, and the like; or microorganisms belonging to Saccharopolyspora, such as Saccharopolyspora taberi, more specifically, Saccharopolyspora taberi JCM 9383t and the like. The PCR may also utilize an oligonucleotide comprising at least about 20 nucleotides of the 5' terminus of the nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, 2, 3, 4, 5, 108, 159, 160, 136, 137, 138, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224, with an oligonucleotide comprising a nucleotide sequence complimentary to at least about 20 nucleotides adjacent to 3' terminus or downstream of the 3' terminus of the nucleotide sequence encoding any one of the amino acid sequences above. The PCR may

be conducted under the conditions described below. On the 5' terminus side of the primer utilized for the PCR as described above, a restriction enzyme recognition sequence may be added.

More specifically for example, a DNA comprising a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 1, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 6, or the like can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces phaeochromogenes IFO12898 and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 51 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 52. Alternatively, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 9 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 1) can be amplified by conducting PCR by utilizing as primers the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 51 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 53.

For example, a DNA comprising a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 2, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 7, or the like can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Saccharopolyspora taberi JCM 9383t and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 61 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 62. Alternatively, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 10 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 2) can be amplified by conducting PCR by utilizing as primers the oligonucleotide comprising the nucleotide

sequence shown in SEQ ID NO: 61 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 63.

For example, a DNA comprising a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 108, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 109, or the like can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces achromogenes IFO 12735 and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 119 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 120. Alternatively, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 110 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 108) can be amplified by conducting PCR by utilizing as primers the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 119 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 121.

For example, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 144 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 159) can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces nogalater IFO 13445 and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 165 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 166.

For example, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 145 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 160) can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces

tsusimaensis IFO 13782 and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 171 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 172.

For example, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 146 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 136) can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces thermocoerulescens IFO14273t and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 177 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 178.

For example, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 147 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 137) can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces glomerochromogenes IFO13673t and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 183 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 184.

For example, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 148 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 138) can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces olivochromogenes IFO 12444 and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 184 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 185.

When utilizing as the template the DNA library in which the chromosomal DNA is introduced into the vector, for example, the present DNA (A) can also be amplified by conducting PCR by utilizing as primers an oligonucleotide comprising a nucleotide sequence selected from a nucleotide sequence encoding any one of the amino acid sequences shown in SEQ ID NO: 1, 2, 3, 4, 5, 108, 159, 160, 136, 137 or 138 (for example, an oligonucleotide comprising a nucleotide sequence of at least about 20 nucleotides of the 5' terminus side of the nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 1) and an oligonucleotide of at least about 20 nucleotides comprising a nucleotide sequence complimentary to the nucleotide sequence adjacent to the DNA insertion site of the vector utilized to construct the library. On side of the 5' terminus of the primer utilized for the PCR as described above, a restriction enzyme recognition sequence may be added.

As the conditions for the such PCR described above, specifically for example, there can be mentioned the condition of maintaining 97℃ for 2 minutes, then repeating for 10 cycles a cycle that includes maintaining 97℃ for 15 seconds, followed by 65℃ for 30 seconds, and then 72℃ for 2 minutes; then conducting for 15 cycles a cycle that includes maintaining 97℃ for 15 seconds, followed by 68℃ for 30 seconds, and followed by 72℃ for 2 minutes (adding 20 seconds to every cycle in turn); and then maintaining 72℃ for 7 minutes. The PCR can utilize a reaction solution of 50μl, containing 50ng of chromosomal DNA, containing 300nM of each of the 2 primers in such pairings described above, containing 5.0μl of dNTP mixture (a mixture of 2.0mM each of the 4 types of dNTPs), containing 5.0μl of 10x Expand HF buffer (containing $MgCl_2$, Roche Molecular Biochemicals Company) and containing 0.75μl of Expand HiFi enzyme mix (Roche Molecular Biochemicals Company).

Alternatively, there can be mentioned the condition of maintaining 97℃ for 2 minutes, then repeating for 30 cycles a cycle that includes 97℃ for 15 seconds, followed by 60℃ for 30 seconds, and followed by 72℃ for 90seconds, and then maintaining the reaction solution at 72℃ for 4 minutes. The PCR can utilize a reaction solution of 50μl containing 250ng of chromosomal DNA, containing 200nM of each of the 2 primers in such pairings described above, containing 5.0μl of dNTP mixture (a mixture of 2.5mM each of the 4 types of dNTPs), 5.0 μl of 10x ExTaq buffer (containing $MgCl_2$, Takara Shuzo Company) and containing 0.5μl of ExTaq Polymerase (Takara Shuzo Company).

Alternatively, for example, oligonucleotides can be designed and prepared for use as primers, based on the nucleotide sequence of a region to which the sequence identity is particularly high in the nucleotide sequence shown in SEQ ID NO: 6, 7, 8 or 109. The present DNA (A) can also be obtained by conducting PCR by utilizing the obtained oligonucleotides as primers and a chromosomal DNA or chromosomal DNA library. The chromosomal DNA or chromosomal DNA library can be prepared as described above from microorganisms belonging to Streptomyces, such as Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseolus, Streptomyces carbophilus, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis and Streptomyces steffisburgensis, and more specifically, Streptomyces phaeochromogenes IFO12898, Streptomyces testaceus ATCC21469, Streptomyces achromogenes IFO 12735, Streptomyces griseolus ATCC11796, Streptomyces carbophilus SANK62585, Streptomyces griseofuscus IFO 12870t, Streptomyces thermocoerulescens IFO 14273t,

Streptomyces nogalater IFO 13445, Streptomyces tsusimaensis IFO 13782, Streptomyces glomerochromogenes IFO 13673t, Streptomyces olivochromogenes IFO 12444, Streptomyces ornatus IFO 13069t, Streptomyces griseus ATCC 10137, Streptomyces griseus IFO 13849T, Streptomyces lanatus IFO 12787T, Streptomyces misawanensis IFO 13855T, Streptomyces pallidus IFO 13434T, Streptomyces roseorubens IFO 13682T, Streptomyces rutgersensis IFO 15875T and Streptomyces steffisburgensis IFO 13446T, and the like; or microorganisms belonging to Saccharopolyspora, such as Saccharopolyspora taberi, more specifically, Saccharopolyspora taberi JCM 9383t and the like. As the "region to which the sequence identity is particularly high in the nucleotide sequence shown in SEQ ID NO: 6, 7, 8 or 109," for example, there is mentioned the region corresponding to the region shown with each of nucleotides 290 to 315, 458 to 485, 496 to 525 or 1046 to 1073 in the nucleotide sequence shown in SEQ ID NO: 6. As the primers designed on the basis of such regions of the nucleotide sequence, for example, there can be mentioned a primer comprising the nucleotide sequence shown in any one of SEQ ID NO: 124 to 129.

SEQ ID NO: 124; based on the nucleotide sequence of the region corresponding to the region shown with the above nucleotides 290 to 315;

SEQ ID NO: 125; based on the nucleotide sequence of the region corresponding to the region shown with the above nucleotides 458 to 485;

SEQ ID NO: 126; based on the nucleotide sequence of the region corresponding to the region shown with the above nucleotides 458 to 485;

SEQ ID NO: 127; based on the nucleotide sequence of the region corresponding to the region shown with the above nucleotides 496 to 525;

SEQ ID NO: 128; based on the nucleotide sequence of the region corresponding to the region shown with the above nucleotides 496 to 525; and

SEQ ID NO: 129; based on the nucleotide sequence of the region corresponding to the region shown with the above nucleotides 1046 to 1073.

For example, a DNA of approximately 800bp is amplified by utilizing as primers the pairing of the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 124 and the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. A DNA of approximately 600bp is amplified by utilizing as primers the pairing of the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 125 and the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. A DNA of approximately 600bp is amplified by utilizing as primers the pairing of the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 126 and the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. A DNA of approximately 580bp is amplified by utilizing as primers the pairing of the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 127 and the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. Further, a DNA of approximately 580bp is amplified by utilizing as primers the pairing of the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 128 and the oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129.

As the conditions for PCR, specifically for example, there is mentioned the condition of maintaining 95℃ for 1 minute; repeating for 30 cycles a cycle that includes maintaining 94℃ for 15 seconds, followed by 60℃ for 30 seconds, and followed by 72℃ for 1 minute; and then maintaining 72℃ for 5 minutes. There can be utilized the reaction solution of 25μl containing 10ng of chromosomal DNA, containing 200nM of each of the 2 primers, containing 0.5μl of dNTP mix (a mixture of 10mM each of the 4 types of dNTPs), containing 5μl of 5xGC genomic PCR reaction buffer, containing 5μl of 5M GC-Melt and containing 0.5μl of Advantage-GC genomic polymerase mix (Clontech

Company).

By recovering the DNA amplified as described above, a DNA comprising a partial nucleotide sequence of the present DNA (A) can be obtained. Next, based on the nucleotide sequence possessed by the obtained "DNA comprising a partial nucleotide sequence of the present DNA (A)", there is designed and prepared an oligonucleotide comprising a partial nucleotide sequence of at least about 20 nucleotides of said nucleotide sequence or an oligonucleotide comprising a nucleotide sequence complimentary to the partial nucleotide sequence of at least about 20 nucleotides of said nucleotide sequence. A DNA comprising a partial nucleotide sequence of the present DNA (A) extended downstream of the 3' terminus or upstream of the 5' terminus of the "DNA comprising a partial nucleotide sequence of the present DNA (A)" obtained as described above can be obtained by conducting PCR. The PCR may utilize as primers a pairing of an oligonucleotide prepared as described above based on the nucleotide sequence of the "DNA comprising a partial nucleotide sequence of the present DNA (A)" and an oligonucleotide of at least about 20 nucleotides comprising a nucleotide sequence of the region adjacent to the DNA insertion site of the vector utilized to construct the above library or an oligonucleotide of at least about 20bp comprising a nucleotide sequence complimentary to such nucleotide sequence thereof. The PCR may, for example, utilize as the template the chromosomal DNA library prepared from the microorganisms which have the ability to convert compound (II) to compound (III), as described above. By connecting such DNA comprising the partial nucleotide sequence of the present DNA (A), there can be obtained the present DNA (A). In such a production method, there can be utilized a commercially available kit, such as the Universal Genome Walker (Clontech Company). Alternatively, the present DNA (A) can be obtained by

conducting PCR by preparing primers based on the full length nucleotide sequence of the present DNA (A) obtained by connecting the partial nucleotide sequences of the present DNA (A) as described above, by utilizing such primers and by utilizing as the template the chromosomal DNA library as described above.

For example, a DNA comprising the nucleotide sequence shown in nucleotides 316 to 1048 of SEQ ID NO: 139 (a partial nucleotide sequence of nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 159), can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces nogalater IFO 13445 and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 124 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. A DNA comprising a nucleotide sequence extended downstream of the 3' terminus or upstream of the 5' terminus thereof is obtained according to the above description based on the nucleotide sequence of the obtained DNA. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 144 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 159 and the nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 149) can be obtained by connecting the resulting DNA.

For example, a DNA comprising the nucleotide sequence shown in nucleotides 364 to 1096 of SEQ ID NO: 140 (a partial nucleotide sequence of nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 160), can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces tsusimaensis IFO 13782 and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO:

124 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. A DNA comprising a nucleotide sequence extended downstream of the 3' terminus or upstream of the 5' terminus thereof is obtained according to the above description based on the nucleotide sequence of the obtained DNA. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 145 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 150 and the nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 160) can be obtained by connecting the resulting DNA.

For example, a DNA comprising the nucleotide sequence shown in nucleotides 295 to 1027 of SEQ ID NO: 141 (a partial nucleotide sequence of nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 136), can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces thermocoerulescens IFO 14273t and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 124 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. A DNA comprising a nucleotide sequence extended downstream of the 3' terminus or upstream of the 5' terminus thereof is obtained according to the above description based on the nucleotide sequence of the obtained DNA. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 146 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 136 and the nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 151) can be obtained by connecting the resulting DNA.

For example, a DNA comprising the nucleotide sequence shown in nucleotides 316 to 1048 of SEQ ID NO: 142 (a partial nucleotide sequence of nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 137), can be prepared by

conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces glomerochromogenes IFO 13673t and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 124 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. A DNA comprising a nucleotide sequence extended downstream of the 3' terminus or upstream of the 5' terminus thereof is obtained according to the above description based on the nucleotide sequence of the obtained DNA. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 147 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 137 and the nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 152) can be obtained by connecting the resulting DNA.

For example, a DNA comprising the nucleotide sequence shown in nucleotides 316 to 1048 of SEQ ID NO: 143 (a partial nucleotide sequence of nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 138), can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces olivochromogenes IFO 12444 and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 124 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. A DNA comprising a nucleotide sequence extended downstream of the 3' terminus or upstream of the 5' terminus thereof is obtained according to the above description based on the nucleotide sequence of the obtained DNA. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 148 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 138 and the nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 153) can be obtained by connecting the resulting DNA.

For example, a DNA comprising the nucleotide sequence shown in nucleotides 289 to 1015 of SEQ ID NO: 232 (a partial nucleotide sequence of nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 222), can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces roseorubens IFO 13682T and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 124 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. A DNA comprising a nucleotide sequence extended downstream of the 3' terminus or upstream of the 5' terminus thereof is obtained according to the above description based on the nucleotide sequence of the obtained DNA. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 242 (containing a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 232 and the nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 252) can be obtained by connecting the resulting DNA.

For example, a DNA comprising the nucleotide sequence shown in nucleotides 289 to 1015 of SEQ ID NO: 234 (a partial nucleotide sequence of nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 224), can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces steffisburgensis IFO 13446T and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 124 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129. A DNA comprising a nucleotide sequence extended downstream of the 3' terminus or upstream of the 5' terminus thereof is obtained according to the above description based on the nucleotide sequence of the obtained DNA. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 244 (containing a nucleotide sequence

encoding the amino acid sequence shown in SEQ ID NO: 234 and the nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 254) can be obtained by connecting the resulting DNA.

The present DNA (A) obtained by utilizing the PCR described above can be cloned into a vector by a method according to conventional genetic engineering methods described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press, "Current Protocols in Molecular Biology" (1987), John Wiley & Sons, Incorporated and the like. Specifically for example, cloning can be conducted by utilizing plasmid vectors such as pBluescriptII of Strategene Company or a plasmid vector contained in the TA Cloning Kit of Invitrogen Company.

Further, the present DNA (A) can be prepared, for example, as described below. First, a nucleotide sequence is designed. The nucleotide sequence encodes an amino acid sequence of a protein encoded by the present DNA (A). The nucleotide sequence has a GC content of at most 60% and at least 40%, preferably at most 55% and at least 45%. The codon usage in the nucleotide sequence encoding the amino acid sequence of the above protein is within the range of plus or minus 4% of the codon usage in genes from the species of a host cell to which the present DNA (A) is introduced. By preparing a DNA having the designed nucleotide sequence according to conventional genetic engineering methods, the present DNA (A) can be obtained.

For example, there can be designed in the way described below, a nucleotide sequence encoding an amino acid sequence (SEQ ID NO: 1) of the present invention protein (A1) and having a GC content of at most 55% and at least 45%, where the codon usage in the nucleotide sequence encoding the amino acid sequence of the above protein

is within the range of plus or minus 4% of the codon usage in genes from soybean. First, for example, the codon usage (Table 22 and Table 23) in the nucleotide sequence (SEQ ID NO: 6) encoding the amino acid sequence of the present invention protein (A1) which can be obtained from Streptomyces phaeochromogenes IFO12898 and soybean codon usage (Table 24 and Table 25) are compared. Based on the result of the comparison, nucleotide substitutions are added to the nucleotide sequence shown in SEQ ID NO: 6, so that the GC content is at most 55% and at least 45% and the codon usage is within the range of plus or minus 4% of the soybean codon usage. As such a nucleotide substitution, there is selected a nucleotide substitution which does not result in an amino acid substitution. For example, the usage of the CTG codon encoding leucine is 1.22% in soybean genes and 7.09% in the nucleotide sequence shown in SEQ ID NO: 6. As such, for example, each of the CTG codons starting from nucleotides 106, 163, 181, 226, 289, 292, 544, 1111, and 1210 of the nucleotide sequence shown in SEQ ID NO: 6 is substituted to CTT codons; each of the CTG codons starting from nucleotides 211, 547 and 1084 is substituted to CTA codons; the CTG codon starting from nucleotide 334 is substituted to a TTA codon; each of the CTG codons starting from nucleotides 664, 718, 733, 772, 835, 1120 and 1141 is substituted to a TTG codon; and the CTG codon starting from nucleotide 787 is substituted to a TTA codon. One sequence of a nucleotide sequence designed in such a way is shown in SEQ ID NO: 214, the codon usage in which is shown in Table 26 and Table 27. In the nucleotide sequence shown in SEQ ID NO: 214, for example, the usage of the CTG codon encoding leucine is 1.71% and is within the range of plus or minus 4% of the codon usage (1.22%) for soybean. The DNA comprising the nucleotide sequence shown in SEQ ID NO: 214 can be prepared by introducing nucleotide substitutions to the DNA having the nucleotide sequence shown in SEQ ID NO: 6, according to site-directed mutagenesis methods described in such as

Sambrook, J., Frisch, E.F., and Maniatis, T.; Molecular Cloning 2nd Edition, Cold Spring Harbor Press. Alternatively, the DNA having the nucleotide sequence shown in SEQ ID NO: 214 can be prepared by a DNA synthesis method employing the PCR described in Example 46 below.

Similarly, the nucleotide sequence shown in SEQ ID NO: 368 is an example of designing a nucleotide sequence encoding the amino acid sequence (SEQ ID NO: 222) of the present invention protein (A23) and having a GC content of at most 55% and at least 45%, where the codon usage in the nucleotide sequence encoding the amino acid sequence of the above protein is within the rage of plus or minus 4% with the codon usage for genes from soybean. Further, the nucleotide sequence shown in SEQ ID NO: 393 is an example of designing a nucleotide sequence encoding the amino acid sequence (SEQ ID NO: 224) of the present invention protein (A25) and having a GC content of at most 55% and at least 45%, where the codon usage in the nucleotide sequence encoding the amino acid sequence of the above protein is within the rage of plus or minus 4% with the codon usage for genes from soybean.

The present DNA (A) obtained in such a way can be cloned into a vector according to conventional genetic engineering methods described in such as Sambrook, J., Frisch, E.F., and Maniatis, T.; "Molecular Cloning 2nd Edition" (1989), Cold Spring Harbor Press; "Current Protocols in Molecular Biology" (1987), John Wiley & Sons, Incorporated, and the like. As the vector, specifically for example, there can be utilized pUC 119 (TaKaRa Shuzo Company), pTVA 118N (Takara Shuzo Company), pBluescript II (Toyobo Company), pCR2.1-TOPO (Invitrogen), pTrc99A (Pharmacia Company), pKK331-1A (Pharmacia Company), and the like.

Further, the nucleotide sequence of the present DNA (A) obtained in such a way can be analyzed by the dideoxy terminator method described in F. Sanger, S. Nicklen,

A.R. Coulson, Proceeding of National Academy of Science U.S.A. (1977) 74:5463-5467.

The ability to metabolize the PPO inhibitory-type herbicidal compound of formula (I) of the present protein (A), which is encoded by the present DNA (A) obtained in such a way described above, can be confirmed with the ability of converting compound (II) to compound (III) as a marker in the way described below. First, as described below, said DNA is inserted into a vector so that it is connected downstream of a promoter which can function in the host cell and that is introduced into a host cell to obtain a transformant. Next, the culture of the transformant or the extract obtained from disrupting the culture is reacted with compound (II) in the presence of an electron transport system containing an electron donor, such as coenzyme NADPH. The reaction products resulting therefrom are analyzed to detect compound (III). In such a way, there can be detected a transformant having the ability of metabolizing compound (II) and producing compound (III), and be determined that such a transformant bears the present DNA (A) encoding the protein having such ability. More specifically for example, there is prepared 30μl of a reaction solution consisting of a 0.1M potassium phosphate buffer (pH 7.0) comprising the culture or extract of the above transformant, an electron donor such as β-NADPH at a final concentration of about 2mM, ferredoxin derived from spinach at a final concentration of about 2mg/ml, ferredoxin reductase at a final concentration of about 0.1U/ml and 3ppm of compound (II) labeled with a radioisotope. The reaction solution is incubated at about 30℃ to 40℃ for 10 minutes to 1 hour. After such incubation, 3μl of 2N HCl and 90μl of ethyl acetate are added, stirred and centrifuged at 8,000g to recover the supernatant. After drying the supernatant in vacuo, the residue is dissolved in ethyl acetate and the obtained solution is developed on a silica gel TLC plate. The TLC plate is analyzed by radio autography. By identifying the spots

corresponding to compound (III) labeled with a radioisotope, there can be confirmed the ability to convert compound (II) to compound (III).

A DNA encoding a protein having the ability to convert compound (II) to compound (III) or a microorganism having such a DNA may be further searched by conducting the hybridizations or PCR as described above, utilizing the present invention DNA (A) or the polynucleotide comprising a partial nucleotide sequence of said DNA or a nucleotide sequence complimentary to the partial nucleotide sequence.

Specifically for example, hybridization as described above is conducted and the DNA to which a probe is hybridized is identified. The hybridization is conducted with the use of the present invention DNA (A) or a polynucleotide comprising a partial nucleotide sequence of the present invention DNA (A) of a nucleotide sequence complimentary to the partial nucleotide sequence as a probe, and genomic DNA derived from a natural microorganism, for example, microorganisms belonging to streptomyces such as Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseolus, Streptomyces carbophilus, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis and Streptomyces steffisburgensis; microorganisms belonging to Saccharopolyspora such as Saccharopolyspora taberi; and the like. As specific examples of DNA which can be utilized as the probe, there can be mentioned a DNA comprising the full length of the nucleotide sequence shown in any one of SEQ ID NO: 6, 7, 8, 109, 139, 140, 141, 142, 143, 225, 226, 227, 228, 229, 230, 231, 232, 233 or 234; a DNA

comprising a nucleotide sequence shown in nucleotides 57 to 730 of the nucleotide sequence shown in SEQ ID NO: 6; a DNA comprising a nucleotide sequence show in nucleotides 21 to 691 of the nucleotide sequence shown in SEQ ID NO: 8; and the like.

Alternatively, PCR can be conducted as described above and the amplified DNA can be detected. The PCR utilizes a polynucleotide comprising a partial nucleotide sequence of the present invention DNA (A) or a nucleotide sequence complimentary to the partial nucleotide sequence. The PCR utilizes as the template genomic DNA derived from a natural microorganism, for example, microorganisms belonging to streptomyces such as Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseolus, Streptomyces carbophilus, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis and Streptomyces steffisburgensis; microorganisms belonging to Saccharopolyspora such as Saccharopolyspora taberi; and the like. As the primers, there can be mentioned primers which were designed, based on the nucleotide sequence of the "region to which the sequence identity is particularly high in the nucleotide sequence shown in SEQ ID NO: 6, 7, 8 or 109" as described above. As more specific examples of the primers, there is mentioned pairings of a primer comprising a nucleotide sequence shown in any one of SEQ ID NO: 124 to 128 and a primer comprising a nucleotide sequence shown in SEQ ID NO: 129.

The DNA detected in such a way is recovered. When the recovered DNA does not contain the full length nucleotide sequence of the present DNA (A), such DNA is utilized and made into a DNA corresponding to the full length nucleotide sequence in a

way described above. The obtained DNA is introduced into a host cell to produce a transformant. The ability to convert compound (II) to compound (III) of the protein encoded by the DNA introduced into the transformant can be evaluated by utilizing the cultureof the obtained transformant and measuring the ability to convert compound (II) to compound (III) in a way described above.

To express the present DNA (A) in a host cell, the present DNA (A) is introduced into the host cell in a position enabling its expression in said cell. By introducing the present DNA (A) into a "position enabling its expression", it means that the present DNA (A) is introduced into a host cell so that it is placed in a position adjacent to a nucleotide sequence directed to transcription and translation from the nucleotide sequence thereof (that is, for example, a nucleotide sequence promoting the production of the present protein (A) and an RNA encoding the present protein (A)).

To introduce the present DNA (A) into the host cell so that it is placed in a position enabling its expression, for example, a DNA in which the present DNA (A) and a promoter functional in the host cell are operably linked is introduced into the host cell. The term "operably linked" here means that a condition in which the present DNA (A) is linked to a promoter so that it is expressed under the control of the promoter, when the DNA is introduced into a host cell.

When the host cell is a microorganism cell, as a functional promoter, for example, there can be mentioned the lactose operon promoter of E. coli, tryptophan operon promoter of E. coli, T7 phage promoter or artificial promoters functional in E. coli such as tac promoter or trc promoter and the like. Further, there may be utilized the promoter originally present upstream of the present DNA (A) in the chromosome of the microorganism belonging to Streptomyces or Saccharopolyspora.

When the host cell is a plant cell, as a functional promoter, for example, there is mentioned T-DNA derived constitutive promoters such as nopaline synthase gene promoter and octopine synthase gene promoter; plant virus-derived promoters such as cauliflower mosaic virus derived 19S and 35S promoters; inducible promoters such as phenylalanine ammonia-lyase gene promoter, chalcone synthase gene promoter and pathogenesis-related protein gene promoter; the plant promoter described in Japanese Unexamined Patent Publication No. 2000-166577. Further, a terminator functional in a plant cell may be connected to the DNA in which the promoter functional in a plant cell and the present DNA (A) are operably linked. In this case, it is generally preferred that the terminator is connected downstream from the present DNA (A). As the funtional terminator, for example, there is mentioned T-DNA derived constitutive terminators such as nopaline synthase gene (NOS) terminator; plant virus derived terminators such as terminators of allium virus GV1 or GV2; the plant terminator described in Japanese Unexamined Patent Publication No. 2000-166577; and the like.

When introducing the present DNA (A) so that the DNA is placed in a position enabling its expression, for example, there can be utilized a DNA having a nucleotide sequence encoding a transit signal to an intracellular organelle, linked upstream of the present DNA (A), so that the reading frames are in frame. By being linked "so that the reading frames are in frame" it means that reading frame of the sequence of the transit signal to an intracellular organelle and the reading frame of the present DNA (A) are connected to form one continuous reading frame. As a transit signal sequence providing the transition and localization of a protein in an intracellular organelle in a plant cell, for example, there can be mentioned a transit signal derived from a cytoplasmic precursor of a protein localizing in the chloroplast of a plant as described in U. S. Pat. No. 5,717,084,

the chimeric sequences formed from the variety of the transit signal sequences described in U. S. Pat. No. RE36449. More specifically, there is mentioned the chloroplast transit peptide derived from the small subunit of ribulose-1,5-bisphosphate carboxylase of soybean, which is obtainable according to the method described in Example 15 below.

Typically, the present DNA (A), the present DNA (A) to which a DNA having a nucleotide sequence encoding a transit signal to an intracellular organelle is connected as described above, or a DNA in which such DNA is operably linked to a promoter functional in the host cell, can each be inserted into a vector usable in a host cell and this is introduced into the host cell. When utilizing a vector already possessing a promoter functional in the host cell, the present DNA (A) may be inserted downstream of a promoter present in the vector so that said promoter and the present DNA (A) can be operably linked.

As the vector, specifically when utilizing E. coli as the host cell, for example, there can be mentioned pUC 119 (TaKaRa Shuzo Company), pTVA 118N (Takara Shuzo Company), pBluescript II (Strategene Company), pCR2.1-TOPO (Invitrogen), pTrc99A (Amersham Pharmacia Biotech Company), pKK331-1A (Amercham Pharmacia Biotech Company), pET11d (Novagen) and the like. By utilizing a vector containing a selective marker (for example, genes conferring resistance to an antibiotic such as a kanamycin resistance gene, neomycin resistance gene, and the like), it is convenient in that the transformant to which the present DNA is introduced can be selected with the phenotype of the selective marker as an indicator.

As the method of introducing the present DNA (A) or a vector containing the present DNA (A) into a host cell, there can be mentioned the method described in Shin

Seikagaku Zikken Kouza (Nippon-Seikagaku-Kai eds., Tokyo Kagaku Dozin), Vol. 17, Biseibutu-Zikken-Hou when the host cell is a microorganism, for example, E. coli, Bacillus subtilis, Bacillus brevis, Pseudomonas sp., Zymomonas sp., lactic acid bacteria, acetic acid bacteria, Staphylococcus sp., Streptomyces sp., Saccharopolyspora sp., or yeast such as Saccharomyces cerevisiae, Schizosaccaromyces ponmbe, fungus such as Aspergillus, and the like. Alternatively, for example, there may be utilized the calcium chloride method described in Sambrook, J., Frisch, E.F., and Maniatis, T.; "Molecular Cloning 2nd edition", Cold Spring Harbor Press (Molecular Biology, John Wiley & Sons, N.Y. (1989) or in "Current Protocols in Molecular Biology" (1987), John Wiley & Sons, Incorporated or the electroporation method described in "Methods in Electroporation: Gene Pulser / E. coli Pulser System", Bio-Rad Laboratories (1993).

The transformant to which the present DNA (A) or the vector containing the present DNA (A) has been introduced, for example, can be selected by selecting for the phenotype of the selective marker contained in the vector to which the present DNA (A) has been inserted as described above as an indicator. Further, whether the transformant contains the present DNA (A) or a vector containing the present DNA (A) can be confirmed by preparing the DNA from the transformant and then conducting with the prepared DNA genetic engineering analysis methods described in, for example, "Molecular Cloning 2nd edition", Cold Spring Harbor Press (Molecular Biology, John Wiley & Sons, N.Y. (1989) (such as confirming restriction enzyme sites, DNA sequencing, southern hybridizations, PCR and the like).

When the host cell is a plant cell, plant types can be mentioned, for example, dicotyledones such as tobacco, cotton, rapeseed, sugar beet, Arabidopsis, canola, flax, sunflower, potato, alfalfa, lettuce, banana, soybean, pea, legume, pine, poplar, apple,

grape, orange, lemon, other citrus fruits, almond, walnut other nuts; monocotyledones such as corn, rice, wheat, barley, rye, oat, sorghum, sugar cane and lawn; and the like. As the cell to which the present DNA (A) is introduced there can be utilized plant tissue, plant body, cultured cells, seeds and the like.

As methods of introducing the present DNA (A) or the vector containing the present DNA (A) into a host cell, there is mentioned methods such as infection with Agrobacterium (Japanese Examined Patent Publication No.2-58917 and Japanese Unexamined Patent Publication No. 60-70080), electroporation into protoplasts (Japanese Unexamined Patent Publication No. 60-251887 and Japanese Unexamined Patent Publication No. 5-68575) or particle gun method (Japanese Unexamined Patent Publication No. 5-508316 and Japanese Unexamined Patent Publication No. 63-258525).

In such cases, for example, the transformant to which the present DNA has been introduced can be selected with the phenotype of a selective marker as an indicator, by introducing into the plant cell at the same time with the vector containing the present DNA (A), a selective maker selected from the hygromycin phosphotransferase gene, neomycin phosphotransferase gene and chloramphenicol acetyltransferase gene. The selective marker gene and the present DNA (A) may be inserted into the same vector and introduced. A vector comprising the selective marker gene and a vector comprising the present DNA (A) may also be introduced at the same time. A transformant to which the present DNA (A) has been introduced may also be selected by culturing with a medium containing the PPO inhibitory-type herbicidal compound of formula (I) and by isolating a clone multipliable therein. Whether the transformant contains the present DNA (A) can be confirmed by preparing the DNA from the transformant and then conducting with the prepared DNA genetic engineering analysis methods described in, for example, "Molecular Cloning 2nd edition", Cold Spring Harbor Press (Molecular Biology, John

Wiley & Sons, N.Y. (1989) (such as confirming restriction enzyme sites, DNA sequencing, southern hybridizations, PCR and the like). The present DNA (A) introduced in the plant cell may be maintained at locations in the cell other than the DNA contained in the nucleus, by being inserted into the DNA contained in intracellular organelles such as the chloroplast.

From the transformed plant cell obtained in such a way, a transgenic plant to which the present DNA (A) has been introduced can be obtained, by regenerating a plant body by the plant cell culturing method described in Shokubutu-Idenshi-Sosa-Manual: Transgenic-Shokubutu-No-Tukurikata (Uchimiya, Kodansha-Scientific, 1990), pp. 27-55. Further, a targeted plant type to which the present DNA (A) has been introduced can be produced by mating the targeted type of plant with the transgenic plant to which the present DNA (A) has been introduced, so that the present DNA (A) is introduced into a chromosome of the targeted type of plant.

Specifically, for example, rice or Arabidopsis having introduced therein the present DNA (A) and expressing the present protein (A) can be obtained by the method described in Model-Shokubutu-No-Jikken-Protocol: Ine, Shiroinunazuna-Hen (Supervisors: Koh SHIMAMOTO and Kiyotaka OKADA, Shujun-sha, 1996), Fourth chapter. Further, there can be obtained a soybean having introduced therein the present DNA (A) and expressing the present protein (A) by an introduction into a soybean somatic embryo with a particle gun according to the method described in Japanese Unexamined Patent Publication No. 3-291501. Likewise, a maize having introduced therein the present DNA (A) and expressing the present protein (A) can be obtained by an introduction into maize somatic embryo with a particle gun according to the method described by Fromm, M.E., et al., Bio/Technology, 8; p 838 (1990). Wheat having

introduced therein the present DNA (A) and expressing the present protein (A) can be obtained by introducing the gene into sterile-cultured wheat immature scutellum with a particle gun according to a conventional method described by TAKUMI et al., Journal of Breeding Society (1995), 44: Extra Vol. 1, p 57. Likewise, barley having introduced therein the present DNA (A) and expressing the present protein (A) can be obtained by an introduction into sterile-cultured barley immature scutellum with a particle gun according to a conventional method described by HAGIO, et al., Journal of Breeding Society (1995), 44; Extra Vol. 1, p 67.

The transformant having introduced therein the present DNA (A) and expressing the present protein (A) can reduce the plant damage by compound (I), by converting said herbicidal compound into a compound of lower herbicidal activity within its cells. Specifically, for example, by spreading the microorganism expressing the present protein (A) to the cultivation area of the desired cultivated plant before sowing seeds of the desired plant, the herbicidal compound remaining in the soil can be metabolized and the damage to the desired plant can be reduced. Further, by getting the desired variety of plant to express the present protein (A), the ability to metabolize the PPO inhibitory-type herbicidal compound of formula (I) to a compound of lower activity is conferred to said plant. As a result, the plant damage from the herbicidal compound in the plant is reduced and resistance to said compound is conferred.

The present protein (A) can be prepared, for example, by culturing a cell comprising the present DNA (A). As such a cell, there is mentioned a microorganism expressing the present DNA (A) and having the ability to produce the present protein (A), such as a microorganism strain isolated from nature comprising the present DNA (A),

mutant strains derived from the natural strain by treatment with agents or ultraviolet rays or the like. More specifically for example, there is mentioned microorganisms belonging to Streptomyces, such as Streptomyces phaeochromogenes IFO12898, Streptomyces testaceus ATCC21469, Streptomyces achromogenes IFO 12735, Streptomyces griseolus ATCC11796, Streptomyces carbophilus SANK62585, Streptomyces griseofuscus IFO 12870t, Streptomyces thermocoerulescens IFO 14273t, Streptomyces nogalater IFO 13445, Streptomyces tsusimaensis IFO 13782, Streptomyces glomerochromogenes IFO 13673t, Streptomyces olivochromogenes IFO 12444, Streptomyces ornatus IFO 13069t, Streptomyces griseus ATCC 10137, Streptomyces griseus IFO 13849T, Streptomyces lanatus IFO 12787T, Streptomyces misawanensis IFO 13855T, Streptomyces pallidus IFO 13434T, Streptomyces roseorubens IFO 13682T, Streptomyces rutgersensis IFO 15875T and Streptomyces steffisburgensis IFO 13446T, and the like; or microorganisms belonging to Saccharopolyspora, such as Saccharopolyspora taberi JCM 9383t and the like. Further, there can be mentioned a transformant in which the present DNA (A) or a vector containing the present DNA (A) has been introduced. Specifically for example, there is mentioned a transformant in which the present DNA (A) operably linked to a tac promoter, trc promoter, lac promoter or t7 phage promoter is introduced into E. coli. As more specific examples, there is mentioned E.coli JM109/pKSN657, E.coli JM109/pKSN657F, E.coli JM109/pKSN923, E.coli JM109/pKSN923F, E.coli JM109/pKSN11796, E.coli JM109/pKSN11796F, E.coli JM109/pKSN671, E.coli JM109/pKSN671F, E.coli JM109/pKSNSCA, E.coli JM109/pKSN646, E.coli JM109/pKSN646F, E.coli JM109/pKSN849AF, E.coli JM109/pKSN1618F, E.coli JM109/pKSN474F, E.coli JM109/pKSN1491AF, E.coli JM109/pKSN1555AF, E.coli JM109/pKSN1584F, E.coli JM109/pKSN1609F and the like, described in the examples described below.

As a medium for culturing the above microorganisms comprising the present DNA (A), there can be utilized any of those employed usually for culturing a microorganism which contains carbon sources and nitrogen sources, organic and inorganic salts as appropriate. A compound which is a precursor to heme, such as aminolevulinic acid, may be added.

As the carbon source, for example, there is mentioned saccharides such as glucose, fructose, sucrose and dextrin; sugar alcohols such as glycerol and sorbitol; and organic acids such as fumaric acid, citric acid and pyruvic acid; and the like. The amount of carbon sources listed above to be added to a medium is usually about 0.1% (w/v) to about 10% (w/v) based on a total amount of the medium.

As the nitrogen source, for example, there is mentioned ammonium salts of inorganic acids such as ammonium chloride, ammonium sulfate and ammonium phosphate; ammonium salts of organic acids such as ammonium fumarate and ammonium citrate; organic nitrogen sources, such as meat extract, yeast extract, malt extract, soybean powder, corn steep liquor, cotton seed powder, dried yeast, casein hydrolysate; as well as amino acids. Among those listed above, ammonium salts of organic acids, organic nitrogen sources and amino acids may mostly be employed also as carbon sources. The amount of nitrogen sources to be added is usually about 0.1% (w/v) to about 10% (w/v) based on the total amount of the medium.

As the inorganic salt, for example, there is mentioned phosphates such as potassium phosphate, dipotassium phosphate, sodium phosphate, disodium phosphate; chlorides such as potassium chloride, sodium chloride, cobalt chloride hexahydrate; sulfates such as magnesium sulfate, ferrous sulfate heptahydrate, zinc sulfate heptahydrate, manganese sulfate trihydrate; and the like. The amount to be added is

usually about 0.0001% (w/v) to about 1% (w/v) based on a total amount of the medium.

In case of culturing a transformant retaining the present DNA (A) connected downstream of a T7 phage promoter and a DNA in which the nucleotide sequence encoding T7 RNA polymerase (λ DE3 lysogen) is connected downstream of a lac UV5 promoter, typically, a small amount of, for example, isopropyl- β -D-thiogalactoside (hereinafter referred to as "IPTG") may be added as an inducer for inducing the production of the present protein (A). IPTG can also be added to the medium in case of culturing a transformant having introduced therein a DNA in which the present DNA (A) is operably linked to a type of promoter which is induced by lactose, such as tac promoter, trc promoter and lac promoter.

A microorganism comprising the present DNA (A) can be cultivated in accordance with a method employed usually to culture a microorganism, including a liquid phase cultivation such as a rotatory shaking cultivation, a reciprocal shaking cultivation, a jar fermentation (Jar Fermenter cultivation) and a tank cultivation; or a solid phase cultivation. When a jar fermenter is employed, aseptic air should be introduced into the Jar Fermenter usually at an aeration rate of about 0.1 to about 2 times culture fluid volume per minute. The temperature at which the cultivation is performed may vary within a range allowing a microorganism to be grown, and usually ranges from about 15℃ to about 40℃, and the pH of the medium ranges from about 6 to about 8. The cultivation time may vary depending on the cultivation conditions, and is usually about 1 day to about 10 days.

The present protein (A) produced by a microorganism comprising the present DNA (A), for example, can be utilized in various forms in the treatment of the PPO

inhibitory-type herbicidal compound of formula (I), such as a culture of a microorganism producing the present protein (A), a cell of a microorganism producing the present protein (A), a material obtained by treating such a cell, a cell-free extract of a microorganism, a crudely purified protein, a purified protein and the like. A material obtained by treating a cell described above includes for example a lyophilized cell, an acetone-dried cell, a ground cell, an autolysate of a cell, an ultrasonically treated cell, an alkali-treated cell, an organic solvent-treated cell and the like. Alternatively, the present protein (A) in any of the various forms described above may be immobilized in accordance with known methods such as a support binding method employing an adsorption onto an inorganic carrier such as a silica gel or a ceramic material, a polysaccharide derivative such as a DEAE-cellulose, a synthesized polymer such as Amberite IRA-935 (Trade Name, manufactured by Rohm and Haas) and the like, and an inclusion method employing an inclusion into a network matrix of a polymer such as a polyacrylamide, a sulfur-containing polysaccharide gel (e.g. carrageenan gel), an alginic acid gel, an agar gel and the like, and then used in the treatment of the herbicidal compound described above.

As methods of purifying the present protein (A) from a culture of a microorganism comprising the present DNA (A), there can be employed conventional methods utilized in a purification of protein. For example, there can be mentioned the following method.

First, cells are harvested from a culture of a microorganism by centrifugation or the like, and then disrupted physically by an ultrasonic treatment, a DYNOMILL treatment, a FRENCH PRESS treatment and the like, or disrupted chemically by utilizing a surfactant or a cell-lyzing enzyme such as lysozyme. From the resultant lysate thus

obtained, insoluble materials are removed by centrifugation, membrane filtration or the like to prepare a cell-free extract, which is then fractionated by any appropriate means for separation and purification, such as a cation exchange chromatography, an anion exchange chromatography, a hydrophobic chromatography, a gel filtration chromatography and the like, whereby purifying the present protein (A). Supporting materials employed in such chromatography include for example a resin support such as cellulose, dextran and agarose connected with a carboxymethyl (CM) group, a diethylaminoethyl (DEAE) group, a phenyl group or a butyl group. A commercially available column already packed with any support such as Q-Sepharose FF, Phenyl-Sepharose HP, PD-10 and HiLoad 26/10 Q Sepharose HP (Trade Name, from Amersham Pharmacia Biotech), TSK-gel G3000SW (Trade Name, TOSOH CORPORATION) may also be employed.

One example of purifying the present protein (A) is given.

Cells of a microorganism producing the present protein (A) are harvested by centrifugation, and then suspended in a buffer such as 0.1M potassium phosphate (pH7.0). The suspension is treated ultrasonically to disrupt the cells, and the resultant lysate thus obtained is centrifuged at about 40,000g for about 30 minutes to obtain a supernatant, which is then centrifuged at 150,000g for about 1 hour to recover the supernatant (the cell-free extract). The obtained cell-free extract is subjected to ammonium sulfate fractionation to obtain the fraction that is soluble in the presence of 45%-saturated ammonium sulfate and precipitates at 55%-saturated ammonium sulfate. After the solvent of the fraction is exchanged with a buffer containing no ammonium sulfate, such as 1M potassium phosphate, utilizing a PD10 column (Amersham Pharmacia Biotech Company), the resulting fraction is loaded, for example, onto a HiLoad 26/10 Q

Sepharose HP column (Amersham Pharmacia Biotech Company). The column is eluted with 20mM bistrispropane with a linear gradient of NaCl to obtain a series of fractions of eluate. The fractions showing activity in converting compound (II) to compound (III) in the presence of an electron transport system containing an electron donor, such as coenzyme NADPH, are recovered. Next, after exchanging the buffer in the fractions by utilizing for example the PD10 column (Amersham Pharmacia Biotech Company), the recovered fractions are loaded onto a Bio-Scale Ceramic, for example, Hydroxyapatite, Type I column CHT10-I (BioRad Company). After washing the column with Buffer A (2mM potassium phosphate buffer containing 1.5mM of $CaCl_2$; pH7.0), the column is eluted with Buffer A with a linear gradient of Buffer B (100mM potassium phosphate buffer containing 0.03mM $CaCl_2$) to obtain a series of fractions of eluate. The fractions showing activity in converting compound (II) to compound (III) in the presence of an electron transport system containing an electron donor, such as coenzyme NADPH, are recovered. After exchanging the buffer in the fractions by utilizing for example the PD10 column (Amersham Pharmacia Biotech Company), the recovered fractions are concentrated by for example ultrafiltration (microcon filter unit microcon-30; Millipore Company). The resulting fraction is injected for example into a HiLoad 16/60 Superdex column 75pg column (Amersham Pharmacia Biotech Company) and eluted with a 0.05M potassium phosphate buffer containing 0.15M NaCl (pH7.0) to obtain a series of fractions of eluate. The fractions showing activity in converting compound (II) to compound (III) in the presence of an electron transport system containing an electron donor, such as coenzyme NADPH, are recovered. The present protein (A) can be purified by a separation with an SDS-PAGE as needed.

By purifying the present invention protein (A) in the way described above,

followed by utilizing the obtained present invention protein (A) as an immune antigen, there can be produced an antibody recognizing the present invention protein (A) (hereinafter sometimes referred to as the "present invention antibody (A)").

Specifically, for example, an animal is immunized with the present protein (A) purified in the way described above, as an antigen. For example, to immunize an animal such as a mouse, hamster, guinea pig, chicken, rat, rabbit, dog and the like, the antigen is administered at least once, utilizing a conventional method of immunization described in, for example, W.H. Newsome, J. Assoc. Off. Anal. Chem. 70(6) 1025-1027 (1987). As the schedule of administration, for example, there is mentioned an administration of 2 or 3 times at 7- to 30-day intervals, preferably, 12- to 16-day intervals. The dose thereof is, for example, from about 0.05mg to 2mg of the antigen for each administration. The administration route may be selected from subcutaneous administration, intracutaneous administration, intraperitoneal administration, intravenous administration, and intramuscular administration and an injection given intravenously, intraabdominally or subcutaneously is a typical administration form. The antigen is typically used after being dissolved in a suitable buffer, for example, sodium phosphate buffer or physiological saline containing at least one type of ordinarily used adjuvant such as complete Freund's adjuvant (a mixture of Aracel A, Bayol F and dead tubercule bacillus), RAS [MPL (monophosphoryl lipid A) + TDM (synthetic trehalose dicorynomycolate) + CWS (cell wall skeleton) adjuvant system] or aluminum hydroxide. However, depending on the administration route or conditions, the adjuvants described above may not be used. The "adjuvant" is a substance which upon administration with the antigen, enhances a immune reaction unspecifically against the antigen. After nurturing the animal administered with the antigen for 0.5 to 4 months, a small amount of blood is sampled from e.g. an ear vein of the animal and measured for antibody titer. When the antibody

titer is increasing, then the antigen is further administered for an appropriate number of times, depending on cases. For example, the antigen may be administered for one more time at a dose of about 100μg to 1000μg. One or two months after the last administration, blood is collected in a usual manner from the immunized animal. By having the blood fractionated by conventional techniques such as precipitation by centrifugation or with ammonium sulfate or with polyethylene glycol, chromatography such as gel filtration chromatography, ion-exchange chromatography and affinity chromatography, and the like, the present invention antibody (A) may be obtained as a polyclonal antiserum. Further, the antiserum may be incubated e.g. at 56 °C for 30 minutes to inactivate the complement system.

Alternatively, a polypeptide comprising a partial amino acid sequence of the present invention protein (A) is synthesized chemically and administered as an immune antigen to an animal, whereby producing the present invention antibody (A). As the amino acid sequence of a polypeptide employed as an immune antigen, an amino acid sequence which has as a low homology as possible with the amino acid sequences of other proteins is selected from amino acid sequences of the present invention protein (A). A polypeptide having a length of 10 amino acids to 15 amino acids consisting of the selected amino acid sequence is synthesized chemically by a conventional method and crosslinked for example with a carrier protein such as Limulus plyhemus hemocyanin using MBS and the like and then used to immunize an animal such as a rabbit as described above.

The resultant present invention antibody (A) is then brought into contact with a test sample, and then a complex of the protein in the test sample with the antibody described above is detected by a conventional immunological method, whereby detecting the present invention protein (A) or a polypeptide comprising a partial amino acid thereof

in the test sample. Specifically, for example, it is possible to evaluate the presence of the present invention protein (A) or to quantify the present invention protein (A) in the examined test sample by a western blot analysis utilizing the present invention antibody (A) as shown in Examples 45 or 73 described below.

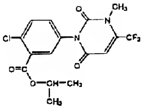
Further, for example, a cell expressing the present protein (A) can be detected, by contacting the present invention antibody (A) with a test cell or a test sample prepared from the test cell followed by detecting a complex of the above antibody and the protein in the test cell or a test sample prepared from the test cell, according to conventional immunology methods. By detecting the cell expressing the present invention protein (A) in such a way, it is also possible to select from a variety of cells, a cell expressing the present invention protein (A). It is also possible to clone or select a clone containing the present invention protein (A) with the use of the present invention antibody (A). For example, a genomic library can be produced by extracting genomic DNA from a cell that expresses the present invention protein (A) followed by inserting the genomic DNA into an expression vector. The genomic library is introduced into a cell. From the obtained cell group, a cell expressing the present invention protein (A) is selected with the use of the present invention antibody (A) in the way described above.

A kit comprising the present invention antibody (A) can be utilized to detect the present invention protein (A) as described above or to analyze, detect or search for a cell expressing the present invention protein (A). The kit of the present invention may contain the reagents necessary for the above analysis methods, other than the present invention antibody (A), and may have such a reagent used together with the present invention antibody (A).

By reacting a PPO inhibitory-type herbicidal compound of formula (I) in the

presence of an electron transport system containing an electron donor, such as coenzyme NADPH, with the present protein (A), the above compound is metabolized and is converted into a compound of lower herbicidal activity. Specifically for example, by reacting compound (II) in the presence of an electron transport system containing an electron donor, such as coenzyme NADPH, with the present protein (A), compound (II) is converted to compound (III), which shows substantially no herbicidal activity. An example of protein (A) in such cases is the present invention protein (A). One variation of the present protein (A) may be utilized and multiple variations may be utilized together.

The compound of formula (I) is a compound having a uracil structure. As specific examples, there can be mentioned compound (II) or a compound of any one of formulas (IV) to (IX) (hereinafter, referred respectively to as compound (IV) to compound (IX)). It is possible to synthesize compound (II) and compound (IX) according to the method described in Japanese Unexamined Patent Publication No. 2000-319264, compound (IV) and compound (V) according to the method described in U. S. Pat. No. 5183492, compound (VI) according to the method described in U. S. Pat. No. 5674810, compound (VII) according to the method described in Japanese Unexamined Patent Publication No. 3-204865, and compound (VIII) according to the method described in Japanese Unexamined Patent Publication No. 6-321941.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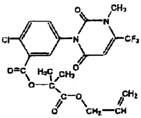
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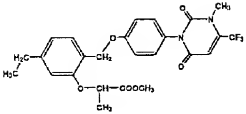
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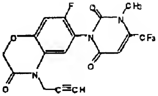
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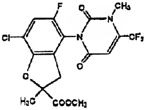
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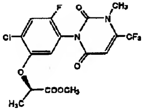
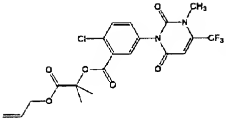

(IX)

Further, as specific examples of the compound of formula (I), there can be mentioned a compound of any one of formulas (X) to (XVII) (hereinafter, respectively referred to as compound (X) to compound (XVII)).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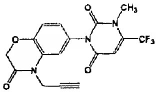
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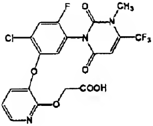
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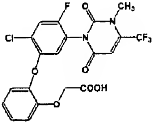
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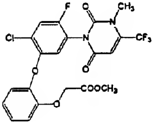
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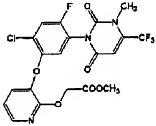
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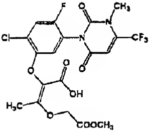
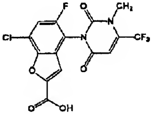

(XVI)


(XVII)

Compounds which can be a substrate of the metabolizing reaction by the present protein (A) can be selected by having the compound present in a reaction in which compound (II) labeled with a radioisotope is reacted with the present protein (A), in the presence of an electron transport system containing an electron donor, such as coenzyme

NADPH, and detecting as a marker the competitive inhibition of the conversion reaction by the present protein (A) of the labeled compound (II) to the labeled compound (III). When assaying for the presence of the competitive inhibition from a test compound, the test compound is typically added to amount to a molar concentration of from 1 to 100 times of the labeled compound (II).

The reaction in which compound (I) is reacted with the present protein (A) can be conducted, for example, in an aqueous buffer containing salts of inorganic acids such as an alkaline metal phosphate such as sodium phosphate and potassium phosphate; or salts of organic acids such as an alkaline metal acetate such as sodium acetate and potassium acetate; or the like. The concentration of the compound of formula (I) in a metabolizing reaction solution is typically at most about 30% (w/v) and preferably about 0.001% (w/v) to 20%(w/v). The amount of the electron transport system containing the electron donor, such as NADPH, or of the present protein (A) may vary, for example, depending on reaction time period. The reaction temperature is chosen from the range of typically from about 10℃ to 70℃, and is preferably about 20℃ to 50℃. The pH of the reaction solution is chosen from the range of typically from about 4 to 12 and is preferably about 5 to 10. The reaction time period may vary as desired, and is typically from about 1 hour to 10 days.

Further, the reaction in which compound (I) is reacted with the present protein (A) can be conducted in a cell comprising the present DNA (A). As the cells comprising the present DNA (A), for example, there is mentioned a microorganism having the ability to express the present DNA (A) and produce the present protein (A), such as, a strain of those microorganisms isolated from nature comprising the present DNA (A), a mutant strain derived from the microorganism strain by treatment with chemicals or ultraviolet

rays, a transformed microorganism cell in which the present DNA (A) or a vector containing the present DNA (A) is introduced into a host cell. Further, there is mentioned a transformed plant cell to which the present DNA (A) is introduced or a cell of a transformed plant to which the present DNA (A) is introduced. In such cases, the compound of formula (I) may be directly applied to a cell comprising the present DNA (A) or may be added to the culturing medium of the cell or the soil coming into contact with the cell, so as to enter the cell. The electron transport system containing the electron donor, such as NADPH, can be the system originally present in the cell and can be added from outside of the cell.

The metabolism of compound (I) by the present protein (A) can be confirmed, for example, by detecting the compound produced by the metabolism of compound (I). Specifically for example, compound (III) produced from metabolizing compound (II) can be detected with the HPLC analysis or TLC analysis, described above.

Further, the metabolism of compound (I) by the present protein (A) can be confirmed on the basis that the herbicidal activity in the reaction solution after compound (I) is reacted with the present protein (A) is comparatively lower than the case in which compound (I) is not reacted with the present protein (A). As a method of testing the herbicidal activity, for example, there is mentioned a method in which the above reaction solutions are applied onto weeds such as barnyardgrass (Echinochloa crus-galli), Blackgrass (Alopercurus myosuroides), Ivyleaf morningglory (Ipomoea hederacea) and Velvetleaf (Abutilon theophrasti), and the herbicidal effects are examined; or a method in which the weeds are cultivated on soil samples to which the above reaction solutions are applied and the herbicidal effects are examined; and the like. Further, there is mentioned a method in which the above reaction solutions may be spotted onto a leaf disk taken

from a plant and the presence of plant damage (whitening) caused by the reaction solution is examined.

Further, the metabolism of compound (I) by the present protein (A) can be confirmed by detecting as a marker, the PPO inhibitory activity in the reaction solution after compound (I) is reacted with the present protein (A), which is comparatively lower than the activity in the reaction solution in which compound (I) is not reacted with the present protein (A). PPO is an enzyme catalyzing the conversion of protoporphyrinogen IX to protoporphyrin IX (hereinafter referred to as "PPIX"). For example, after adding the above reaction solutions to a reaction system of PPO, protoporphyrinogen IX, which is a substrate of PPO, is added and incubated for about 1 to 2 hours at 30℃ in the dark. Subsequently, the amount of PPIX in each of the incubated solutions is measured, utilizing an HPLC or the like. When the amount of PPIX in system to which the reaction solution after compound (I) is reacted with the present protein (A) is added is more than the amount of PPIX in system to which the reaction solution in which compound (I) is not reacted with the present protein (A) is added, it is determined that compound (I) had been metabolized by the present protein (A). As PPO, there may be utilized a protein purified from plants and the like or chloroplast fraction extracted from a plant. When utilizing the chloroplast fractions, aminolevulinic acid may be utilized in the reaction system of PPO, instead of protoporphyrinogen IX. Aminolevulinic acid is the precursor of protoporphyrinogen IX in the chlorophyll-heme biosynthesis pathway. A more specific example is given in Example 42 below.

By reacting with the present protein (A) in such a way, there can be conducted a treatment of the PPO inhibitory-type herbicidal compound of formula (I), which results in metabolization and conversion of the compound to a compound of lower herbicidal

activity. The plant damage from said compound can be reduced by the treatment in which said compound which was sprayed onto the cultivation area of a plant, specifically for example, the compound which was sprayed onto the cultivation area of a plant and remains in plant residue or the soil or the like, is reacted with the present protein (A).

As the "electron transport system containing the electron donor" which can be utilized to react compound (I) with the present protein (A), for example, there can be mentioned a system containing NADPH, ferredoxin and ferredoxin-NADP$^+$ reductase.

As a method of presenting the "electron transport system containing an electron donor" in a system for reacting compound (I) with the present protein (A), for example, there is mentioned a method of adding to the above reaction system, NADPH, ferredoxin derived from a plant such as spinach and ferredoxin-NADP$^+$ reductase derived from a plant such as spinach. Further, there may be added to said reaction system, a fraction containing a component functional for the electron transport system in the reaction system of the present protein (A), which may be prepared from a microorganism such as E. coli. In order to prepare such a fraction, for example, after cells are harvested from a culture of a microorganism by centrifugation or the like, the cells are disrupted physically by an ultrasonic treatment, a DYNOMILL treatment, a FRENCH PRESS treatment and the like, or disrupted chemically by utilizing a surfactant or a cell-lyzing enzyme such as lysozyme. From the resultant lysate thus obtained, insoluble materials are removed by centrifugation, membrane filtration or the like to prepare a cell-free extract The cell-free extract as is can be utilized in exchange of the above ferredoxin as the fraction containing a component functional for the electron transport system in the reaction system of the present protein (A). Further, when a system which can transport an electron from an electron donor to the present protein (A) is present in such a cell, as with the case in

which the reaction of the present protein (A) with compound (I) is conducted in a cell such as a microorganism or a plant cell, no electron transport system may be newly added.

As the ferredoxin, for example, there can be utilized a ferredoxin derived from microorganisms belonging to Streptomyces, such as Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseolus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis and Streptomyces steffisburgensis, and more specifically, Streptomyces phaeochromogenes IFO12898, Streptomyces testaceus ATCC21469, Streptomyces achromogenes IFO 12735, Streptomyces griseolus ATCC11796, Streptomyces thermocoerulescens IFO 14273t, Streptomyces nogalater IFO 13445, Streptomyces tsusimaensis IFO 13782, Streptomyces glomerochromogenes IFO 13673t, Streptomyces olivochromogenes IFO 12444, Streptomyces ornatus IFO 13069t, Streptomyces griseus ATCC 10137, Streptomyces griseus IFO 13849T, Streptomyces lanatus IFO 12787T, Streptomyces misawanensis IFO 13855T, Streptomyces pallidus IFO 13434T, Streptomyces roseorubens IFO 13682T, Streptomyces rutgersensis IFO 15875T and Streptomyces steffisburgensis IFO 13446T, and the like; or microorganisms belonging to Saccharopolyspora, such as Saccharopolyspora taberi, more specifically, Saccharopolyspora taberi JCM 9383t and the like (hereinafter, sometimes collectively referred to as the "present protein (B)"). Specifically for example, there can be mentioned a ferredoxin selected from the protein group below (hereinafter, sometimes referred to as the "present invention protein (B)"):

<protein group>

(B1) a protein comprising an amino acid sequence shown in SEQ ID NO: 12 (hereinafter, sometimes referred to as the "present invention protein (B1)");

(B2) a protein comprising an amino acid sequence shown in SEQ ID NO: 13 (hereinafter, sometimes referred to as the "present invention protein (B2)");

(B3) a protein comprising an amino acid sequence shown in SEQ ID NO: 14 (hereinafter, sometimes referred to as the "present invention protein (B3)");

(B4) a protein comprising an amino acid sequence shown in SEQ ID NO: 111 (hereinafter, sometimes referred to as the "present invention protein (B4)");

(B5) a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

(B6) a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

(B7) a protein comprising an amino acid sequence shown in SEQ ID NO: 149 (hereinafter, sometimes referred to as the "present invention protein (B7)");

(B8) a protein comprising an amino acid sequence shown in SEQ ID NO: 150 (hereinafter, sometimes referred to as the "present invention protein (B8)");

(B9) a protein comprising an amino acid sequence shown in SEQ ID NO: 151 (hereinafter, sometimes referred to as the "present invention protein (B9)");

(B10) a protein comprising an amino acid sequence shown in SEQ ID NO: 152 (hereinafter, sometimes referred to as the "present invention protein (B10)");

(B11) a protein comprising an amino acid sequence shown in SEQ ID NO: 153 (hereinafter, sometimes referred to as the "present invention protein (B11)");

(B12) a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with any one of the amino acid sequence shown in SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, or SEQ ID NO: 253 or an amino acid sequence having at least 90% sequence identity with any one of the amino acid sequence shown in SEQ ID NO: 150, SEQ ID NO: 252 or SEQ ID NO: 254;

(B13) a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with any of the nucleotide sequence encoding an amino acid sequence shown in SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253 or SEQ ID NO: 254;

(B14) a protein comprising the amino acid sequence shown in SEQ ID NO: 245;

(B15) a protein comprising the amino acid sequence shown in SEQ ID NO: 247;

(B16) a protein comprising the amino acid sequence shown in SEQ ID NO: 248;

(B17) a protein comprising the amino acid sequence shown in SEQ ID NO: 249;

(B18) a protein comprising the amino acid sequence shown in SEQ ID NO: 250;

(B19) a protein comprising the amino acid sequence shown in SEQ ID NO: 251;

(B20) a protein comprising the amino acid sequence shown in SEQ ID NO: 252;

(B21) a protein comprising the amino acid sequence shown in SEQ ID NO: 253; and

(B22) a protein comprising the amino acid sequence shown in SEQ ID NO: 254.

A DNA encoding the present protein (B) (hereinafter, sometimes referred to as the "present DNA (B)") can be obtained according to conventional genetic engineering

methods described in Molecular Cloning: A Laboratory Manual 2nd edition (1989), Cold Spring Harbor Laboratory Press; Current Protocols in Molecular Biology (1987), John Wiley & Sons, Incorporated and the like, based on the nucleotide sequences encoding the amino acid sequences of the present invention protein (B) shown in SEQ ID NO: 12, 13, 14, 111, 149, 150, 151, 152, 153, 245, 247, 248, 249, 250, 251, 252, 253 or 254.

As the DNA encoding the present invention protein (B) (hereinafter, sometimes collectively referred to as the "present invention DNA (B)"), there is mentioned

a DNA encoding a protein comprising an amino acid sequence shown in SEQ ID NO: 12 (hereinafter, sometimes referred to as the "present invention DNA (B1)");

a DNA encoding a protein comprising an amino acid sequence shown in SEQ ID NO: 13 (hereinafter, sometimes referred to as the "present invention DNA (B2)");

a DNA encoding a protein comprising an amino acid sequence shown in SEQ ID NO: 14 (hereinafter, sometimes referred to as the "present invention DNA (B3)");

a DNA encoding a protein comprising an amino acid sequence shown in SEQ ID NO: 111 (hereinafter, sometimes referred to as the "present invention DNA (B4)");

a DNA encoding a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

a DNA encoding a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

a DNA encoding a protein comprising an amino acid sequence shown in SEQ ID NO: 149 (hereinafter, sometimes referred to as the "present invention DNA (B7)");

a DNA encoding a protein comprising an amino acid sequence shown in SEQ ID NO: 150 (hereinafter, sometimes referred to as the "present invention DNA (B8)");

a DNA encoding a protein comprising an amino acid sequence shown in SEQ ID NO: 151 (hereinafter, sometimes referred to as the "present invention DNA (B9)");

a DNA encoding a protein comprising an amino acid sequence shown in SEQ ID NO: 152 (hereinafter, sometimes referred to as the "present invention DNA (B10)");

a DNA encoding a protein comprising an amino acid sequence shown in SEQ ID NO: 153 (hereinafter, sometimes referred to as the "present invention DNA (B11)");

a DNA encoding a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, or SEQ ID NO: 253 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 150, SEQ ID NO: 252 or SEQ ID NO: 254;

a DNA encoding a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253 or SEQ ID NO: 254;

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 245;

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 247;

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 248;

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 249;

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 250;

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 251;

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 252;

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 253; and

a DNA encoding a protein comprising the amino acid sequence shown in SEQ ID NO: 254.

As more specific examples of the present invention DNA (B), there can be mentioned a DNA comprising a nucleotide sequence shown in any one of SEQ ID NO: 15, 16, 17, 112, 154, 155, 156, 157, 158, 255, 257, 258, 259, 260, 261, 262, 263 or 264, or a DNA comprising a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence shown in any one of SEQ ID NO: 15, 16, 17, 112, 154, 155, 156, 157, 158, 255, 257, 258, 259, 260, 261, 262, 263 or 264.

Such DNA can be prepared by conducting methods in which PCR is conducted with DNA comprising a partial nucleotide sequence of the nucleotide sequences thereof as primers or hybridization methods in which such DNA is used as probes, according to the conditions described above in the methods of preparing the present DNA (A).

Specifically for example, a DNA comprising a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 12 or a DNA comprising the nucleotide sequence shown in SEQ ID NO: 15, can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces phaeochromogenes IFO12898 and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 105 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 53.

Further, a DNA comprising a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 13 or a DNA comprising the nucleotide sequence shown in SEQ ID NO: 16, can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Saccharopolyspora taberi JCM 9383t and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 106 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 63.

Further, a DNA comprising a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 14 or a DNA comprising the nucleotide sequence shown in SEQ ID NO: 17, can be prepared by conducting PCR by utilizing as the template the chromosomal DNA or chromosomal DNA library prepared from Streptomyces testaceus ATCC21469 and by utilizing as primers an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 107 and an oligonucleotide comprising the nucleotide sequence shown in SEQ ID NO: 72.

Further, for example, the present invention DNA (B) can be obtained by hybridizing with a chromosomal DNA library, a DNA consisting of about at least 20 nucleotides comprising the nucleotides sequence encoding an amino acid sequences shown in any one of SEQ ID NO: 12, 13, 14, 111, 149, 150, 151, 152 or 153, as a probe

under the conditions described above, followed by detecting and recovering the DNA which bound specifically with said probe. The chromosomal DNA library can be prepared as described above from microorganisms belonging to Streptomyces, such as Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis and Streptomyces steffisburgensis, and more specifically, Streptomyces phaeochromogenes IFO12898, Streptomyces testaceus ATCC21469, Streptomyces achromogenes IFO 12735, Streptomyces thermocoerulescens IFO 14273t, Streptomyces nogalater IFO 13445, Streptomyces tsusimaensis IFO 13782, Streptomyces glomerochromogenes IFO 13673t, Streptomyces olivochromogenes IFO 12444, Streptomyces ornatus IFO 13069t, Streptomyces griseus ATCC 10137, Streptomyces griseus IFO 13849T, Streptomyces lanatus IFO 12787T, Streptomyces misawanensis IFO 13855T, Streptomyces pallidus IFO 13434T, Streptomyces roseorubens IFO 13682T, Streptomyces rutgersensis IFO 15875T and Streptomyces steffisburgensis IFO 13446T, and the like; or microorganisms belonging to Saccharopolyspora, such as Saccharopolyspora taberi, more specifically, Saccharopolyspora taberi JCM 9383t and the like. As specific examples of the DNA which can be utilized as such probes, there is mentioned a DNA comprising a nucleotide sequence shown in any one of SEQ ID NO: 15, 16, 17, 112, 154, 155, 156, 157, 158, 255, 257, 258, 259, 260, 261, 262, 263 or 264; DNA comprising a partial nucleotide sequence of such nucleotide sequences; or a DNA comprising a nucleotide sequence complimentary to said partial nucleotides sequences.

To express the present DNA (B) with a host cell, for example, a DNA in which the present DNA (B) and a promoter functional in a host cell are operably linked is prepared according to conventional genetic engineering methods described in "Molecular Cloning: A Laboratory Manual 2nd edition (1989)", Cold Spring Harbor Laboratory Press; "Current Protocols in Molecular Biology (1987)", John Wiley & Sons, Incorporated and the like, and is introduced into a host cell. Whether the obtained transformant contains the present DNA (B) can be confirmed by preparing the DNA from the transformant and then conducting with the prepared DNA genetic engineering analysis methods described in, for example, "Molecular Cloning 2nd edition", Cold Spring Harbor Press (Molecular Biology, John Wiley & Sons, N.Y. (1989) (such as confirming restriction enzyme sites, DNA sequencing, southern hybridizations, PCR and the like).

The present DNA (B) and the present DNA (A) can be expressed in the same cell, by introducing into a cell comprising the present DNA (A), the DNA in which the present DNA (B) and a promoter functional in a host cell are operably linked.

The present protein (B) can be prepared, for example, by culturing a cell comprising the present DNA (B). As such a cell, there is mentioned a microorganism expressing the present DNA (B) and having the ability to produce the present protein (B), such as microorganism strain isolated from nature comprising the present DNA (B), mutant strains derived from said natural strain by treatment with agents or ultraviolet rays or the like. For example, there is mentioned microorganisms belonging to Streptomyces, such as Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseolus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces

olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis and Streptomyces steffisburgensis, and more specifically, Streptomyces phaeochromogenes IFO12898, Streptomyces testaceus ATCC21469, Streptomyces achromogenes IFO 12735, Streptomyces griseolus ATCC11796, Streptomyces thermocoerulescens IFO 14273t, Streptomyces nogalater IFO 13445, Streptomyces tsusimaensis IFO 13782, Streptomyces glomerochromogenes IFO 13673t, Streptomyces olivochromogenes IFO 12444, Streptomyces ornatus IFO 13069t, Streptomyces griseus ATCC 10137, Streptomyces griseus IFO 13849T, Streptomyces lanatus IFO 12787T, Streptomyces misawanensis IFO 13855T, Streptomyces pallidus IFO 13434T, Streptomyces roseorubens IFO 13682T, Streptomyces rutgersensis IFO 15875T and Streptomyces steffisburgensis IFO 13446T, and the like; or microorganisms belonging to Saccharopolyspora, such as Saccharopolyspora taberi, more specifically, Saccharopolyspora taberi JCM 9383t and the like. Further, there can be mentioned a transformant in which the present DNA (B) has been introduced. Specifically for example, there is mentioned a transformant in which the present DNA (B) operably linked to a tac promoter, trc promoter, lac promoter or T7 phage promoter has been introduced into E. coli. As more specific examples, there is mentioned E.coli JM109/pKSN657FD, E.coli JM109/pKSN923FD, E.coli JM109/pKSN671FD and the like described in the examples described below.

The microorganism comprising the present DNA (B) can be cultivated in accordance with a method employed usually to culture a microorganism, and more specifically, conducted according to the conditions described above in the methods of culturing the microorganism comprising the present DNA (A).

The present protein (B) produced by the microorganism comprising the present DNA (B), for example, can be utilized in various forms in reaction system of the present protein (A), such as a culture of a microorganism producing the present protein (B), a cell of a microorganism producing the present protein (B), a material obtained by treating such a cell, a cell-free extract of a microorganism, a crudely purified protein, a purified protein and the like. A material obtained by treating a cell described above includes for example a lyophilized cell, an acetone-dried cell, a ground cell, an autolysate of a cell, an ultrasonically treated cell, an alkali-treated cell, an organic solvent-treated cell and the like. Alternatively, the present protein (B) in any of the various forms described above may be immobilized in accordance with known methods such as a support binding method employing an adsorption onto a synthesized polymer and the like, and an inclusion method employing an inclusion into a network matrix of a polymer, and then used in the reaction system of the present protein (A).

As methods of purifying the present protein (B) from a culture of a microorganism comprising the present DNA (B), there can be employed conventional methods utilized in a purification of protein. For example, there can be mentioned the following method.

First, cells are harvested from a culture of a microorganism by centrifugation or the like, and then disrupted physically by an ultrasonic treatment and the like, or disrupted chemically by utilizing a surfactant or a cell-lyzing enzyme such as lysozyme. From the resultant lysate thus obtained, insoluble materials are removed by centrifugation, membrane filtration or the like to prepare a cell-free extract, which is then fractionated by any appropriate means for separation and purification, such as a cation exchange chromatography, an anion exchange chromatography, a hydrophobic chromatography, a

gel filtration chromatography and the like, whereby purifying the present protein (B). By separation of the fraction thus obtained with an SDS-PAGE, the present protein (B) can be further purified.

The function of the present protein (B) as ferredoxin can be confirmed as a function of electron transporter from ferredoxin-$NADP^+$ reductase to the present protein (A) in the reaction system in which compound (I) is reacted with the present protein (A). Specifically for example, there can be a confirmation by adding the present protein (B) with NADPH, ferredoxin-$NADP^+$ reductase and the present protein (A) to the reaction system in which compound (I) is reacted with the present protein (A), followed by detecting the conversion of compound (II) to compound (III).

In the method of controlling weeds of the present invention, compound (I) is applied to the cultivation area of a plant expressing the present protein (A). Such a plant may express one variation of the present protein (A) or may express multiple variations of the present protein (A). As the present protein (A), for example, there may be mentioned the present invention protein (A). Plants expressing the present protein (A) can be obtained as a transgenic plant to which the present DNA (A) has been introduced. Such introduction involves introducing the present DNA (A) into a plant cell in the way described above so that the DNA is placed in a position enabling its expression, followed by regenerating a plant from the obtained transformed cell. The present DNA (A) introduced into the plant cell may have linked upstream therefrom, a nucleotide sequence encoding a transit signal to an intracellular organelle, so that the reading frames are in frame.

The plant having introduced therein the present DNA (A) and expressing the

present protein (A) metabolizes compound (I), within its cells, into a compound of lower herbicidal activity. As a result, the plant damage from the herbicidal compound in the plant is reduced and resistance to said compound is conferred. As such, the plant having introduced therein the present DNA (A) and expressing the present protein (A) can grow well even in a case in which compound (I) is applied to a cultivation area thereof. Weeds other than the plant having introduced therein the present DNA (A) and expressing the present protein (A) can be removed effectively by cultivating said plant and applying the above herbicidal composition to the cultivation area. It is possible to improve the yield of the above plant, improve the quality, reduce the amount of utilized herbicide and save labor.

The evaluation of resistance of the cell expressing the present protein (A) to the compound of formula (I) or a herbicidal composition comprising said compound can be carried out by contacting the cell expressing the gene encoding the present protein (A) with said compound or said herbicidal composition and evaluating the degree of damage to the cell.

Specifically, to evaluate the resistance of a microorganism cell expressing the present protein (A) to compound (I) or the herbicidal composition comprising compound (I), a transformed E. coli expressing plant PPO and the present protein (A) may be prepared. Such preparation involves additionally introducing the present DNA (A) into, for example, a transformed E. coli which can be utilized to evaluate PPO activity inhibition and has been described in Japanese patent application No. 11-102534,.more specifically, a transformed E. coli in which a plant PPO gene described in U. S. Pat. No. 5939602 or the like is operably introduced into the E. coli BT3 strain and expressing the PPO gene. The E. coli BT3 strain has a defect in PPO gene and has no proliferation

ability, as described in F. Yamamoto, H. Inokuti, H. Ozaki, (1988) Japanese Journal of Genetics, Vol. 63, pg. 237-249. The resistance to the compound or the herbicidal composition can be evaluated by cultivating the resulting transformed E. coli with shaking for about 18 to 24 hours at 37℃ in a liquid culture medium containing compound (I) or the herbicidal composition comprising said compound in an amount of from 0 to 1.0 ppm and measuring the proliferation of said transformed E. coli with an optical density at 600nm. As the present protein (A), for example, there can be mentioned the present invention protein (A).

As a method of evaluating the degree of resistance of a plant expressing the present protein (A) to the compound of formula (I) or a herbicidal composition comprising said compound, there is mentioned a method of applying the herbicidal composition to the plant and measuring the degree of growth of the plant. For more quantitative confirmation, for example, first, pieces of leaves of the plant are dipped in aqueous solutions containing compound (I) at various concentrations, or the aqueous solutions of compound (I) are sprayed on pieces of leaves of the plant, followed by allowing to stand on an agar medium in the light at room temperature. After several days, chlorophyll is extracted from the plant leaves according to the method described by Mackenney, G., J. Biol. Chem., 140; p 315 (1941) to determine the content of chlorophyll. Specifically for example, leaves of the plant are taken and are split equally into 2 pieces along the main vein. The herbicidal composition is spread onto the full surface of one of the leaf pieces. The other leaf piece is left untreated. These leaf pieces are placed on MS medium containing 0.8% agar and allowed to stand in the light at room temperature for 7 days. Then, each leaf piece is ground with pestle and mortar in 5 ml of 80% aqueous acetone solution to extract chlorophyll. The extract liquid is diluted 10 fold with 80% aqueous acetone solution and the absorbance is measured at 750 nm, 663nm and 645 nm to

calculate total chlorophyll content according to the method described by Mackenney G., J. Biol. Chem. (1941) 140, p 315. The degree of resistance to compound (I) can be comparatively evaluated by showing in percentiles the total chlorophyll content of the treated leaf piece with the total chlorophyll content of the untreated leaf piece. As the present protein (A), for example, the present invention protein (A) can be mentioned.

Based on the above method of evaluating the degree of resistance to compound (I) or a herbicidal composition comprising compound (I), there can be selected a plant or a plant cell showing a resistance to compound (I) or a herbicidal composition comprising compound (I). For example, there is selected a plant where no damage can be seen from spraying compound (I) or a herbicidal composition comprising the compound to the cultivation area of the plant, or plant cell that continuously grows through culturing in the presence of compound (I). Specifically, for example, soil is packed into a plastic pot having, for example, a diameter of 10cm and a depth of 10cm. Seeds of the plant are sowed and cultivated in a greenhouse. An emulsion is prepared by mixing 5 parts of a herbicidal composition comprising compound (I), 6 parts of sorpol3005X (Toho chemicals) and 89 parts of xylene. A certain amount thereof was diluted with water containing 0.1% (v/v) of a sticking agent at a proportion of 1000L for 1 hectare and is spread uniformly with a spray-gun onto the all sides of the foliage from above the plant cultivated in the above pot. After cultivating the plants for 16 days in a greenhouse, the damage to the plants is investigated. The plants in which the damage is not observed or the plants in which the damage is reduced may be selected. Further, progeny plants can be obtained by mating such selected plants.

EXAMPLES

The present invention is explained in more detail with the Examples below, but the present invention is not limited to such examples.

The HPLC for content analysis in Examples 1, 41 and 42 and fraction purification of the compound was conducted under the conditions shown below.

(HPLC analysis condition 1)

column: SUMIPAX ODS211 (Sumika Chemical Analysis Service)

column temperature: 35℃

flow rate: 1ml/minute

detection wave length: UV254nm

eluent A: 0.01% TFA aqueous solution

eluent B: acetonitrile

elution conditions: The sample is injected to the column equilibrated with a solvent mixture of 90% of eluent A and 10% eluent B. The solvent mixture of 90% of eluent A and 10% eluent B is then flowed for 5 minutes. This is followed by flowing a solvent mixture of eluent A and eluent B for 20 minutes, while increasing the proportion of eluent B from 10% to 90%. A solvent mixture of 10% of eluent A and 90% of eluent B is then flowed for 8 minutes.

Example 1 The Metabolism of Compound (II) by a Microorganism

(1) Metabolism of compound (II)

The various microorganisms shown in Tables 1 and 2 were grown in ISP2 agar medium (1.0%(w/v) malt extract, 0.4%(w/v) yeast extract, 0.4% (w/v) glucose, 2.0%(w/v) agar, pH 7.3). A "loopful" of the each microorganism was added to TGY

medium (0.5%(w/v) tryptone, 0.5%(w/v) yeast extract, 0.1%(w/v) glucose, 0.01%(w/v) KH_2PO_4, pH 7.0) and incubated with shaking at 30℃ for 2 to 4 days. One-tenth milliliter (0.1ml) of the obtained culture was incubated with shaking in 3 ml of sporulation medium (0.1%(w/v) of meat extract, 0.2%(w/v) tryptose, 1% glucose, pH 7.1) containing compound (II) at 100ppm for 7 to 8 days at 30℃. Fifty microliters (50μl) of 2N HCl was added to the resulting culture and this was extracted with 3ml of ethyl acetate. The obtained ethyl acetate layer was analyzed on the HPLC. The concentration of compound (II) was reduced (column retention time of 23.9 minutes) and new peaks were detected for compounds at retention times of 21.6 minutes and 22.2 minutes (each referred to as metabolite (I) and metabolite (II)). The results are shown in Tables 1 and 2.

Table 1

strain of the microorganism	concentration of compound (II) (ppm)	peak area of metabolite (I) ($x10^4$)	peak area of metabolite (II) ($x10^4$)
Streptomyces cacaoiasoensis IFO13813	77.8	3.43	3.57
Streptomyces griseofuscus IFO12870t	49.5	7.96	9.86
Streptomyces ornatus IFO13069t	65.3	4.30	5.00
Streptomyces thermocoerulescens IFO14273t	51.7	7.47	9.16
Streptomyces roseochromogenes ATCC13400	81.9	0.71	0.82
Streptomyces lavendulae ATCC11924	89.6	1.02	1.50
Streptomyces griseus ATCC10137	65.6	6.19	1.30
Streptomyces griseus ATCC11429	30.3	12.8	15 6
Streptomyces griseus ATCC12475	51.1	0.52	2.27
Streptomyces griseus ATCC15395	75.2	1.91	2.26
Streptomyces erythreus ATCC11635	54.6	4.94	6.05
Streptomyces scabies IFO3111	88.3	3.28	4.40
Streptomyces griseus IFO3102	22.6	14.4	18.5
Streptomyces catenulae IFO12848	85.3	3.81	1.59
Streptomyces kasugaensis ATCC15714	92.4	1.08	0.91
Streptomyces rimosus ATCC10970	70.9	2.30	2.87
Streptomyces achromogenes IFO12735	0.0	15.9	21.8
Streptomyces lydicus IFO13058	62.0	5.48	6.69

Table 2

strain of the microorganism	concentration of compound (II) (ppm)	peak area of metabolite (I) ($x10^4$)	peak area of metabolite (II) ($x10^4$)
Streptomyces phaeochromogenes IFO12898	46.4	8.28	10.5
Streptomyces afghaniensis IFO12831	80.6	2.54	3.59
Streptomyces hachijoensis IFO12782	83.9	4.99	2.91
Streptomyces argenteolus var. toyonakensis ATCC21468	13.0	14.9	19.2
Streptomyces testaceus ATCC21469	18.4	11.6	14.4
Streptomyces purpurascens ATCC25489	70.9	5.37	6.11
Streptomyces griseochromogenes ATCC14511	53.9	3.00	3.97
Streptomyces kasugaensis IFO13851	66.3	12.1	12.6
Streptomyces argenteolus var.toyon ATCC21468t	90.1	2.75	3.01
Streptomyces roseochromogenes ATCC13400t	71.8	4.66	4.00
Streptomyces nogalater IFO13445	12.8	21.9	24.9
Streptomyces roseochromogenus ATCC21895	74.2	4.14	5.87
Streptomyces fimicarius ATCC21900	46.5	8.33	11.3
Streptomyces chartreusis ATCC21901	61.1	3.70	3.94
Streptomyces globisporus subsp. globisporus ATCC21903	79.9	2.86	2.52
Streptomyces griseolus ATCC11796	0	14.4	19.9
Saccharopolyspora taberi JCM9383T	82.9	5.83	7.71
Streptomyces sp. SANK62585	54.6	2.30	3.44

(2) Structure Determination of the metabolite (I) and metabolite (II)

A frozen stock of Streptomyces griseus ATCC11429 was added to 3ml of a

microorganism culture medium (0.7%(w/v) polypeptone, 0.5%(w/v) yeast extract, 1.0%(w/v) of glucose, 0.5%(w/v) of K_2HPO_4, pH7.2) and incubated with shaking in a test tube overnight to obtain a pre-culture. Such pre-culture was added to 300ml of the microorganism medium containing compound (II) at a concentration of 100ppm. This was divided into 100 small test tubes at 3ml each and incubated with shaking at 30℃ for 6 days. After 250ml of such culture was adjusted to a pH2 by adding HCl, this was extracted with 250ml of ethyl acetate. The solvents were removed from the ethyl acetate layer. The residue was dissolved in 3ml of acetone and spotted to a silica gel TLC plate (TLC plate silica gel $60F_{254}$, 20cm x 20xm, 0.25mm thickness, Merck Company). The TLC plate was developed with 5:7:1 (v/v/v) mixture of toluene, formic acid and ethyl formate. The Rf value around 0.58 of the silica gel was taken. Such contents of the TLC plate were extracted with acetone. The acetone was removed from the extraction layer. The residue was dissolved in 10ml of acetonitrile and fractionated with a HPLC. The fractions containing only metabolite (I) and metabolite (II) were recovered to obtain 3.7mg of metabolites (hereinafter referred to as "metabolite A").

Mass spectrometry analysis of metabolite A was conducted. Metabolite A had a mass that was 14 smaller than compound (II). Further, from H-NMR analysis, it was determined that metabolite (A) was a compound having the structure shown in formula (III).

(3) Herbicidal activity test of compound (III)

Soil was packed into a round plastic pot having a diameter of 10cm and depth of 10cm. Barnyardgrass, Blackgrass, Ivyleaf morningglory were seeded and cultivated in a greenhouse for 10 days. Five (5) parts of the test compound, 6 parts of sorpol3005X (Toho Chemical Company) and 89 parts of xylene were well mixed to produce an

emulsion. A certain amount thereof was diluted with water containing 0.1% (v/v) of a sticking agent at a proportion of 1000L for 1 hectare and was spread uniformly with a spray-gun onto the all sides of the foliage from above the plant cultivated in the above pot. After cultivating the plants for 16 days in a greenhouse, the herbicidal activity of the test compound was investigated. The results are shown in Table 3.

Table 3

test compounds	concentration (g/ha)	Herbicidal Activity		
		Barnyardgrass	Blackgrass	Ivyleaf Morningglory
compound (II)	500	10	10	10
	125	10	10	10
compound (III)	500	0	0	0
	125	0	0	0

Soil was packed into a round plastic pot having a diameter of 10cm and depth of 10cm. Barnyardgrass, Blackgrass, Ivyleaf morningglory were seeded. Five (5) parts of the test compound, 6 parts of sorpol3005X (Toho Chemical Company) and 89 parts of xylene were well mixed to produce an emulsion. A certain amount thereof was diluted with water containing 0.1% (v/v) of a sticking agent at a proportion of 1000L for 1 hectare and was spread uniformly with a spray-gun onto the surface of the soil. After cultivating the plants for 19 days in a greenhouse, the herbicidal activity was investigated. The results are shown in Table 4.

Table 4

test compounds	concentration (g/ha)	Herbicidal Activity		
		Barnyardgrass	Blackgrass	Ivyleaf Morningglory
compound (II)	500	10	10	10
compound (III)	500	0	0	0

In the above Tables 3 and 4, the strength of the herbicidal activity is shown stepwise as 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10. The number "0" represents situations in which the condition of sprouting or vegetation at the time of examination of the plant utilized for the test was compared with and showed totally or substantially no difference with that of the untreated application. The number "10" represents situations in which the plant completely withered or the sprouting or vegetation was completely suppressed.

Example 2 Preparation of the Present Invention Protein (A1)

(1) Preparation of the crude cell extract

A frozen stock of Streptomyces phaeochromogenes IFO12898 was added to 100ml of A medium (0.1%(w/v) glucose, 0.5%(w/v) tryptone, 0.5%(w/v) yeast extract, 0.1%(w/v) of dipotassium hydrogenphosphate, pH7.0) in a 500ml triangular flask and incubated with rotary shaking at 30℃ for 1 day to obtain a pre-culture. Eight milliliters (8ml) of the pre-culture was added to 200ml of A medium and was incubated with rotary shaking in 500ml a baffled flask at 30℃ for 2 days. Cell pellets were recovered by centrifuging (3,000g, 5 min.) the resulting culture. These cell pellets were suspended in 100ml of B medium (1%(w/v) glucose, 0.1% beef extract, 0.2%(w/v) tryptose) containing compound (II) at 100ppm and were incubated with reciprocal shaking in a 500ml Sakaguchi flask for 16 hours at 30℃. Cell pellets were recovered by centrifuging (3,000g, 5 min.) 10L of the resulting culture. The resulting cell pellets were washed twice with 1L of 0.1M potassium phosphate buffer (pH7.0) to provide 162g of the cell pellets.

These cell pellets were suspended in 0.1M potassium phosphate buffer (pH7.0) at 2ml for 1g of the cell pellets, and 1mM PMSF, 5mM benzamidine HCl, 1mM EDTA and

1mM of dithiotritol were added thereto. A cell lysate solution was obtained by disrupting twice repetitively the suspension with a French press (1000kg/cm^2) (Ohtake Seisakusho). After centrifuging the cell lysate solution (40,000xg, 30 minutes), the supernatant was recovered and centrifuged for 1 hour at 150,000xg to recover the supernatant (hereinafter referred to as the "crude cell extract").

(2) Determination of the ability of converting compound (II) to compound (III)

There was prepared 30μl of a reaction solution of 0.1M potassium phosphate buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2.4mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 0.5mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 18μl of the crude cell extract recovered in Example 2(1). The reaction solution was maintained at 30℃ for a hour. Further, there was prepared and maintained similarly a reaction solution having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B and component C. Three microliters (3μl) of 2N HCl and 90 μl of ethyl acetate were added and mixed into each of the reaction solutions after the maintenance. The resulting reaction solutions were centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof was spotted to a TLC plate (TLC plate silica gel $60F_{254}$ 20cm x 20cm, 0.25 thick, Merck Company). The TLC plate was developed with a 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents were then allowed to evaporate. The TLC plate was exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging

plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). The results are shown in Table 5.

Table 5

Reaction components					spot of compound (III)
component A	component B	component C	crude cell extract	compound (II) labeled with ^{14}C	
+	+	+	−	+	−
+	+	+	+	+	+
−	+	+	+	+	−
+	−	−	+	+	−

(3) Fractionation of the crude cell extract

Ammonium sulfate was added to the crude cell extract obtained in Example 2(1) to amount to 45% saturation. After stirring in ice-cooled conditions, the supernatant was recovered by centrifugation for 10 minutes at 12,000xg. After adding ammonium sulfate to the obtained supernatant to amount to 55% saturation and stirring in ice-cooled conditions, a pellet was recovered by centrifuging for 10 minutes at 12,000xg. The pellet was dissolved with 27.5ml of 20mM bistrispropane buffer (pH7.0). This solution was subjected to a PD10 column (Amersham Pharmacia Company) and eluted with 20mM of bistrispropane buffer (pH7.0) to recover 38.5ml of fractions containing proteins (hereinafter referred to as the "45-55% ammonium sulfate fraction").

(4) Isolation of the present invention protein (A1)

The 45-55% ammonium sulfate fraction prepared in Example 2(3) was injected into a HiLoad26/10 Q Sepharose HP column (Amersham Pharmacia Company). Next, after flowing 106ml of 20mM bistrispropane buffer (pH7.0) into the column, 20mM

bistrispropane buffer was flown with a linear gradient of NaCl (gradient of NaCl was 0.001415M/minute, range of NaCl concentration was from 0M to 0.375M, flow rate was 3ml/minute) to fraction recover 25ml of fractions eluting at the NaCl concentration of from 0.21M to 0.22M. Further, the recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) and eluted with 20mM bistrispropane buffer (pH7.0) to recover the fractions containing protein.

The recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) with the elution with Buffer A (2mM potassium phosphate buffer containing 1.5mM of NaCl, pH 7.0), in order to recover the fractions containing protein. Next, the fractions were injected into a Bio-Scale Ceramic Hydroxyapatite Type I column CHT10-I (BioRad Company). Thirty milliliters (30ml) of Buffer A was flown into the column. Subsequently, Buffer A was flown with a linear gradient of Buffer B (100mM potassium phosphate buffer containing 0.03mM of NaCl; the linear gradient started at 100% Buffer A to increase to 50% Buffer B over a 100 minute period, flow rate was 2ml/minute) to fraction recover the fractions eluting at a Buffer B concentration of from 17% to 20%. Further, the recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) and eluted with 0.05M potassium phosphate buffer (pH7.0) to recover the fractions containing protein.

The recovered fractions were concentrated 20 fold using an ultrafilter membrane (Microcon YM-30, Millipore Company) and injected into a HiLoad 16/60 Superdex 75pg column (Amersham Pharmacia Biotech Company). Fifty millimolar (50mM) potassium phosphate buffer containing 0.15M of NaCl (pH7.0) was flown (flow rate 1ml/minute) into the column. The elution was fractioned at 2ml each. The fractions eluting at the elution volumes of from 56ml to 66ml were each fraction recovered. The protein contained in each of the fractions was analyzed with a 10%-20% SDS-PAGE.

Instead of the crude cell extract in the reaction solution described in Example 2(2), the recovered fractions were added and maintained in the presence of component A, component B, component C and compound (II) labeled with ^{14}C, similarly to Example 2(2). The reaction solutions after the maintenance were TLC analyzed to examine the intensity of the spots corresponding to compound (III) labeled with ^{14}C. The protein moving to the position to 47kDa in the above SDS-PAGE was observed to have its fluctuations in the concentrations of the bands of the fractions added in turn to be parallel with the fluctuations of the intensity of the spots corresponding to compound (III). Said protein was recovered from the SDS-PAGE gel and was subjected to an amino acid sequence analysis with a protein sequencer (Applied Biosystems Company, Procise 494HT, pulsed liquid method). As a result, the amino acid sequence shown in SEQ ID NO: 18 was provided. Further, after digesting the above protein with trypsin, the obtained digestion material was analyzed on a mass spectrometer (ThermoQuest Company, Ion Trap Mass Spectrometer LCQ, column: LC Packings Company PepMap C18 75μm x 150mm, solvent A: 0.1%HOAc-H_2O, solvent B: 0.1% HOAc-methanol, gradient: a linear gradient starting at an elution with a mixture of 95% of solvent A and 5% of solvent B and increasing to a concentration of 100% of solvent B over 30 minutes, flow rate: 0.2μl/minute). As a result, the sequence shown in SEQ ID NO: 19 was provided.

Example 3 Obtaining the Present Invention DNA (A1)

(1) Preparation of the chromosomal DNA of Streptomyces phaeochromogenes IFO12898

Streptomyces phaeochromogenes IFO12898 was incubated with shaking at 30℃ for 1 day to 3 days in 50ml of YEME medium (0.3%(w/v) yeast extract, 0.5%(w/v)

bacto-peptone, 0.3%(w/v) malt extract, 1.0%(w/v) glucose, 34%(w/v) sucrose and 0.2%(v/v) 2.5M $MgCl_2 \cdot 6H_2O$). The cells were recovered. The obtained cells were suspended in YEME medium containing 1.4%(w/v) glycine and 60mM EDTA and further incubated with shakking for a day. The cells were recovered from the culture medium. After washing once with distilled water, it was resuspended in buffer (100mM Tris-HCl (pH8.0), 100mM EDTA, 10mM NaCl) at 1ml per 200mg of the cells. Two hundred micrograms per milliliter (200μg/ml) of egg-white lysozyme were added. The cell suspension was incubated with shaking at 30℃ for a hour. Further, 0.5% of SDS and 1mg/ml of Proteinase K was added. The cell suspension was incubated at 55℃ for 3 hours. The cell suspension was extracted twice with mixture of phenol, chloroform and isoamyl alcohol to recover each of the aqueous layers. Next, there was one extraction with mixture of chloroform and isoamyl alcohol to recover the aqueous layer. The chromosomal DNA was obtained by ethanol precipitation from the aqueous layer.

(2) Preparation of the chromosomal DNA library of Streptomyces phaeochromogenes IFO12898

Nine hundred forty-three nanograms (943ng) of the chromosomal DNA prepared in Example 3(1) were digested with 1unit of restriction enzyme Sau3AI at 37℃ for 60 minutes. The obtained digestion solution was separated with 0.7% agarose gel electrophoresis. The DNA of about 2.0kbp was recovered from the gel. The DNA was purified with a Prep-A-Gene^R DNA purification kit (Bio-Rad company) according to the instructions attached to said kit to obtain 10μl of the solution containing the target DNA. A microliter (1μl) of the DNA solution, 98ng of plasmid vector pUC118 digested with restriction enzyme BamHI and treated with dephosphorylation and 11μl of the I solution from Ligation Kit Ver. 2 (Takara Shuzo Company) were mixed and incubated overnight

at 16°C. E coli DH5 α was transformed utilizing 5μl of the ligation solution. The E. coli was cultured with shaking overnight at 30°C. From the obtained culture medium, the E. coli was recovered. The plasmid was extracted to provide the chromosomal DNA library.

(3) **Isolation of the present invention DNA (A1)**

PCR was conducted by utilizing as the template the chromosomal DNA prepared in Example 3(1) (Fig. 1). As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 35 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 36 (hereinafter referred to as "primer paring 1"). The nucleotide sequence shown in SEQ ID NO: 35 was designed based on a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 18. Further, the nucleotide sequence shown in SEQ ID NO: 36 was designed based on a nucleotide sequence complimentary to the nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 19. The PCR reaction solution amounted to 25μl by adding the 2 primers each amounting to 200nM, 250ng of the above chromosomal DNA, 0.5μl of dNTP mix (a mixture of 10mM of each of the 4 types of dNTP; Clontech Company), 5μl of 5xGC genomic PCR reaction buffer (Clontech Company), 1.1μl of 25mM $Mg(OAc)_2$, 5μl of 5M GC-Melt (Clontech Company) and 0.5μl of Advantage-GC genomic polymerase mix (Clontech Company) and distilled water. The reaction conditions of the PCR were after maintaining 95°C for 1 minute, repeating 30 cycles of a cycle that included maintaining 94°C for 15 seconds, followed by 60°C for 30 seconds, followed by 72°C for 1 minute, and then maintaining 72°C for 5 minutes. After the maintenance, the reaction solution was subjected to 4% agarose gel electrophoresis. The gel area containing the DNA of about 150bp was recovered. The DNA was purified from the recovered gel by utilizing QIAquick gel extraction kit

(Qiagen Company) according to the attached instructions. The obtained DNA was ligated to the TA cloning vector pCR2.1 (Invitrogen Company) according to the instructions attached to said vector and was introduced into E. Coli TOP10F'. The plasmid DNA was prepared from the obtained E. coli transformant, utilizing QIAprep Spin Miniprep Kit (Qiagen Company). A sequencing reaction was conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the -21M13 primer (Applied Biosystems Japan Company) and M13Rev primer (Applied Biosystems Japan Company). The sequencing reaction utilized the obtained plasmid DNA as the template. The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). As a result, the nucleotide sequence shown in nucleotides 36 to 132 of the nucleotide sequence shown in SEQ ID NO: 9 was provided. Said nucleotide sequence encoded the amino acid sequence shown in amino acids 12 to 23 of the amino acid sequence shown in SEQ ID NO: 18. In this regard, it was expected that said DNA encoded a part of the present invention protein (A1).

Next, PCR was conducted similar to the above with Advantage-GC genomic polymerase mix (Clontech Company) and by utilizing the chromosomal DNA prepared in Example 3(2) as the template. There was utilized as primers, a pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 37 with an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 38 (hereinafter referred to as the "primer pairing 2") or a pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 39 with an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 40 (hereinafter referred to as the "primer pairing 3").

Next, there was amplified by PCR a DNA having a nucleotide sequence in which

the 3' terminus extends past the nucleotide shown as nucleotide 132 of the nucleotide sequence shown in SEQ ID NO: 9. The PCR was conducted by utilizing as the template solution the reaction solution obtained with the use of primer pairing 2 and by utilizing as primers a pairing of the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 41 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 38 (hereinafter referred to as "primer pairing 4"). Similarly, there was amplified by PCR a DNA having a nucleotide sequence in which the 5' terminus extends past the nucleotide shown as nucleotide 36 of the nucleotide sequence shown in SEQ ID NO: 9. The PCR was conducted by utilizing as the template solution the reaction solution obtained with the use of primer pairing 3 and by utilizing as primers a pairing of the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 42 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 40 (hereinafter referred to as "primer pairing 5"). The 2kbp DNA amplified with the use of primer pairing 4 and the 150bp DNA amplified with the use of primer pairing 5 are cloned into TA cloning vector pCR2.1, similar to the above. Plasmid DNA was prepared from the obtained E. coli transformant, utilizing QIAprep Spin Miniprep Kit (Qiagen Company). A sequencing reaction was conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the -21M13 primer (Applied Biosystems Japan Company), M13Rev primer (Applied Biosystems Japan Company) and the oligonucleotides shown in SEQ ID NO: 43-50. The sequencing reaction utilized the obtained plasmid DNA as the template. The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). As a result of sequencing the nucleotide sequence of the 2kbp DNA amplified by utilizing primer pairing 4, the nucleotide sequence shown in nucleotides 133 to 1439 of the nucleotide sequence shown in SEQ ID NO: 9 was provided. Further, as a result

of sequencing the nucleotide sequence of the 150bp DNA amplified by utilizing primer pairing 5, the nucleotide sequence shown in nucleotides 1 to 35 of the nucleotide sequence shown in SEQ ID NO: 9 was provided. As a result of connecting the obtained nucleotide sequences, the nucleotide sequence shown in SEQ ID NO: 9 was obtained. Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 6) consisting of 1227 nucleotides (inclusive of the stop codon) and encoding a 408 amino acid residue as well as a nucleotide sequence (SEQ ID NO: 15) consisting of 201 nucleotides (inclusive of the stop codon) and encoding a 66 amino acid residue. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 1) encoded by the nucleotide sequence shown in SEQ ID NO: 6 was calculated to be 45213Da. Further, the amino acid sequence encoded by said nucleotide sequence contained the amino acid sequence (SEQ ID NO: 18) determined from the amino acid sequencing of from the N terminus of the present invention protein (A1) and the amino acid sequence (SEQ ID NO: 19) determined from the amino acid sequencing of the trypsin digestion fragments with the mass spectrometer analysis. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 12) encoded by the nucleotide sequence shown in SEQ ID NO: 15 was calculated to be 6818Da.

Example 4 Expression of the Present Invention Protein (A1) in E. coli

(1) Production of a transformed E. coli having the present invention protein (A1)

PCR was conducted by utilizing as a template the chromosomal DNA prepared from Streptomyces phaeochromogenes IFO12898 in Example 3(1) and by utilizing Expand High Fidelity PCR System (Roche Molecular Biochemicals Company). As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 51 and an oligonucleotide having the nucleotide

sequence shown in SEQ ID NO: 52 (hereinafter referred to as "primer pairing 19") or a pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 51 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 53 (hereinafter referred to as "primer pairing 20"). The PCR reaction solution amounted to 50μl by adding the 2 primers each amounting to 300nM, 50ng of the above chromosomal DNA, 5.0μl of dNTP mix (a mixture of 2.0mM of each of the 4 types of dNTP), 5.0μl of 10x Expand HF buffer (containing $MgCl_2$) and 0.75μl of Expand HiFi enzyme mix and distilled water. The reaction conditions of the PCR were after maintaining 97℃ for 2 minutes; repeating 10 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 65℃ for 30 seconds and followed by 72℃ for 2 minutes; then conducting 15 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 68℃ for 30 seconds and followed by 72℃ for 2 minutes (wherein 20 seconds was added to the maintenance at 72℃ for each cycle); and then maintaining 72℃ for 7 minutes. After the maintenance, the reaction solution was subjected to 1% agarose gel electrophoresis. The gel area containing the DNA of about 1.2kbp was recovered from the gel which was subjected the reaction solution utilizing primer pairing 19. The gel area containing the DNA of about 1.5kbp was recovered from the gel which was subjected the reaction solution utilizing primer pairing 20. The DNA were purified from each of the recovered gels by utilizing QIAquick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA were ligated to the TA cloning vector pCR2.1 (Invitrogen Company) according to the instructions attached to said vector and were introduced into E. Coli TOP10F'. The plasmid DNA were prepared from the obtained E. coli transformants, utilizing QIAprep Spin Miniprep Kit (Qiagen Company). Sequencing reactions were conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit,

utilizing as primers the -21M13 primer (Applied Biosystems Japan Company), M13Rev primer (Applied Biosystems Japan Company), the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 43 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 46. The sequencing reactions utilized the obtained plasmid DNA as the template. The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). Based on the results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 6 was designated as pCR657 and the plasmid having the nucleotide sequence shown in SEQ ID NO: 9 was designated as pCR657F.

Furthermore, the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 134 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 135 were annealed together to provide a linker (Fig. 47). Plasmid pKSN24R2 (Akiyoshi-ShibaTa M. et al., Eur. J. Biochem. 224: P335(1994)) was digested with HindIII and XmnI. The linker was inserted into the obtained DNA of about 3kb. The obtained plasmid was designated as pKSN2 (Fig. 4).

Next, each of plasmids pCR657 and pCR657F was digested with restriction enzymes NdeI and HindIII. The digestion products were subjected to agarose gel electrophoresis. The gel area containing a DNA of about 1.2kbp was cut from the gel subjected to the digestion products of pCR657. The gel area containing a DNA of about 1.5kbp was cut from the gel subjected to the digestion products of pCR657F. The DNA were purified from each of the recovered gels by utilizing QIAquick gel extraction kit (Qiagen Company) according to the attached instructions. Each of the obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated with ligation kit Ver.1 (Takara Shuzo Company) according to the instructions attached to said kit and introduced into E. Coli JM109. The plasmid DNA were prepared from the obtained E. coli transformants. The structures thereof were analyzed. The plasmid containing the

nucleotide sequence shown in SEQ ID NO: 6, in which the DNA of about 1.2kbp encoding the present invention protein (A1) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN657. Further, the plasmid containing the nucleotide sequence shown in SEQ ID NO: 9, in which the DNA of about 1.5kbp encoding the present invention protein (A1) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN657F. Each of the above plasmids of pKSN657 and pKSN657F were introduced into E. coli JM109. The obtained E. coli transformants were designated, respectively, JM109/pKSN657 and JM109/pKSN657F. Further, plasmid pKSN2 was introduced into E. coli JM109. The obtained E. coli transformant was designated as JM109/pKSN2.

(2) Expression of the present invention protein (A1) in E. coli and recovery of said protein

E. coli JM109/pKSN657, JM109/pKSN657F and JM109/pKSN2 were each cultured overnight at 37℃ in 10ml of TB medium (1.2%(w/v) tryptone, 2.4%(w/v) of yeast extract, 0.4%(w/v) of glycerol, 17mM potassium dihydrogenphosphate, 72mM dipotassium hydrogenphosphate) containing 50μg/ml of ampicillin. A milliliter (1ml) of the obtained culture medium was transferred to 100ml of TB medium containing 50μg/ml of ampicillin and cultured at 26℃. When OD660 reached about 0.5, 5- aminolevulinic acid was added to the final concentration of 500μM, and the culturing was continued. Thrity (30) minutes thereafter, IPTG was added to a final concentration of 1mM, and there was further culturing for 17 hours.

The cells were recovered from each of the culture mediums, washed with 0.1M tris-HCl buffer (pH7.5) and suspended in 10ml of the above buffer containing 1mM PMSF. The obtained cell suspensions were subjected 6 times to a sonicator (Sonifier

(Branson Sonic Power Company)) at 3 minutes each under the conditions of output 3, duty cycle 30%, in order to obtain cell lysate solutions. After centrifuging the cell lysate solutions (1,200xg, 5 minutes) the supernatants were recovered and centrifuged (150,000xg, 70 minutes) to recover supernatant fractions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN657 is referred to as "E. coli pKSN657 extract ", the supernatant fraction obtained from E. coli JM109/pKSN657F is referred to as "E. coli pKSN657F extract", and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract "). A microliter (1μl) of the above supernatant fractions was analyzed on a 15% to 25% SDS-PAGE and stained with Coomasie Blue (hereinafter referred to as "CBB"). As a result, notably more intense bands were detected in both E. coli pKSN657 extract and E. coli pKSN657F extract than the E. coli pKSN2 extract, at the electrophoresis locations corresponding to the molecular weight of 47kDa. A more intense band was detected in E. coli pKSN657F extract than E. coli pKSN657 extract. It was shown that E. coli JM109/pKSN657F expressed the present invention protein (A1) to a higher degree than E. coli JM109/pKSN657.

(3) Detection of the ability to convert compound (II) to compound (III)

Reaction solutions of 30μl were prepared and maintained for 1 hour at 30℃. The reaction solutions consisted of a 0.1M potassium phosphate buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 0.2mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 18μl of the supernatant fraction recovered in Example 4(2). Further, there were prepared and maintained similarly reaction solutions having no addition of at least one component

utilized in the composition of the above reaction solution, selected from component A, component B and component C. Three microliters (3μl) of 2N HCl and 90 μl of ethyl acetate were added and stirred into each of the reaction solutions after the maintenance. The resulting reaction solutions were centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof was spotted to a TLC plate (TLC plate silica gel $60F_{254}$ 20cm x 20cm, 0.25 thick, Merck Company). The TLC plate was developed with a 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents were then allowed to evaporate. The TLC plate was exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). The results are shown in Table 6.

Table 6

Reaction components					spot of
component A	component B	component C	E. coli extract	compound (II) labeled with ^{14}C	compound (III)
+	+	+	−	+	−
+	+	+	pKSN2	+	−
+	+	+	pKSN657	+	+
−	+	+	pKSN657	+	−
+	−	+	pKSN657	+	−
+	+	−	pKSN657	+	+
+	+	+	pKSN657F	+	+
−	+	+	pKSN657F	+	−
+	−	+	pKSN657F	+	−
+	+	−	pKSN657F	+	+

Example 5 Preparation of the Present Invention Protein (A2)

(1) Preparation of the crude cell extract

A frozen stock of Saccharopolyspora taberi JCM 9383t was added to 10ml of A medium (0.1%(w/v) glucose, 0.5%(w/v) tryptone, 0.5%(w/v) yeast extract, 0.1%(w/v) of dipotassium hydrogenphosphate, pH7.0) in a 10ml test tube and incubated with shaking at 30℃ for 1 day to obtain a pre-culture. Eight milliliters (8ml) of the pre-culture was added to 200ml of A medium and was revolve cultured in 500ml a baffled flask at 30℃ for 2 days. Cell pellets were recovered by centrifuging (3,000xg, 10 min.) 10L of the resulting culture. These cell pellets were suspended in 100ml of B medium (1%(w/v) glucose, 0.1% beef extract, 0.2%(w/v) tryptose) containing compound (II) at 100ppm and were incubated with reciprocal shaking in a 500ml Sakaguchi flask for 20 hours at 30℃. Cell pellets were recovered by centrifuging (3,000xg, 10 min.) 10L of the resulting culture. The resulting cell pellets were washed twice with 1L of 0.1M potassium phosphate buffer (pH7.0) to provide 119g of the cell pellets.

These cell pellets were suspended in 0.1M potassium phosphate buffer (pH7.0) at 2ml for 1g of the cell pellets. A millimolar of (1mM) PMSF, 5mM of benzamidine HCl, 1mM of EDTA, 3μg/ml of leupeptin, 3μg/ml of pepstatin and 1mM of dithiotritol were added. A cell lysate solution was obtained by disrupting twice repetitively the suspension with a French press (1000kg/cm^2) (Ohtake Seisakusho). After centrifuging the cell lysate solution (40,000xg, 30 minutes), the supernatant was recovered and centrifuged for 1 hour at 150,000xg to recover the supernatant (hereinafter referred to as the "crude cell extract").

(2) Determination of the ability of converting compound (II) to compound (III)

There was prepared 30μl of a reaction solution of 0.1M potassium phosphate

buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2.4mM of β -NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 0.5mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 18μl of the crude cell extract recovered in Example 5(1). The reaction solution was maintained at 30℃ for a hour. Further, there was prepared and maintained similarly a reaction solution having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B and component C. Three microliters (3μl) of 2N HCl and 90 μl of ethyl acetate were added and mixed into each of the reaction solutions after the maintenance. The resulting reaction solutions were centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof was spotted to a TLC plate (TLC plate silica gel $60F_{254}$ 20cm x 20cm, 0.25 thick, Merck Company). The TLC plate was developed with a 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents were then allowed to evaporate. The TLC plate was exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). The results are shown in Table 7.

Table 7

Reaction components					spot of compound (III)
component A	component B	component C	crude cell extract	compound (II) labeled with ^{14}C	
+	+	+	−	+	−
+	+	+	+	+	+
−	+	+	+	+	−
+	−	−	+	+	−

(3) Fractionation of the crude cell extract

Ammonium sulfate was added to the crude cell extract obtained in Example 5(1) to amount to 45% saturation. After stirring in ice-cooled conditions, the supernatant was recovered by centrifuging for 10 minutes at 12,000xg. After adding ammonium sulfate to the obtained supernatant to amount to 55% saturation and stirring in ice-cooled conditions, a pellet was recovered by centrifuging for 10 minutes at 12,000xg. The pellet was dissolved with 32.5ml of 20mM bistrispropane buffer (pH7.0). This solution was subjected to a PD10 column (Amersham Pharmacia Company) and eluted with 20mM of bistrispropane buffer (pH7.0) to recover 45.5ml of fractions containing proteins (hereinafter referred to as the "45-55% ammonium sulfate fraction").

(4) Isolation of the present invention protein (A2)

The 45-55% ammonium sulfate fraction prepared in Example 5(3) was injected into a HiLoad26/10 Q Sepharose HP column (Amersham Pharmacia Company). Next, after flowing 100ml of 20mM bistrispropane buffer (pH7.0) into the column, 20mM bistrispropane buffer was flown with a linear gradient of NaCl (gradient of NaCl was 0.004M/minute, range of NaCl concentration was from 0M to 0.5M, flow rate was 8ml/minute) to fraction recover 30ml of fractions eluting at the NaCl concentration of

from 0.25M to 0.26M. Further, the recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) and eluted with 20mM bistrispropane buffer (pH7.0) to recover the fractions containing protein.

The recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) with the elution with Buffer A (2mM potassium phosphate buffer containing 1.5mM of NaCl, pH 7.0), in order to recover the fractions containing protein. Next, the fractions were injected into a Bio-Scale Ceramic Hydroxyapatite Type I column CHT10-I (BioRad Company). Twenty milliliters (20ml) of Buffer A was flown into the column. Subsequently, Buffer A was flown with a linear gradient of Buffer B (100mM potassium phosphate buffer containing 0.03mM of NaCl; the linear gradient started at 100% Buffer A to increase to 50% Buffer B over a 100 minute period, flow rate was 2ml/minute) to fraction recover 10ml of fractions eluting at a Buffer B concentration of from 23% to 25%. Further, the recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) and eluted with 0.05M potassium phosphate buffer (pH7.0) to recover the fractions containing protein.

The recovered fractions were concentrated to about 770µl using an ultrafilter membrane (Microcon YM-30, Millipore Company) and injected into a HiLoad 16/60 Superdex 75pg column (Amersham Pharmacia Biotech Company). Fifty millimolar (50mM) potassium phosphate buffer containing 0.15M of NaCl (pH7.0) was flown (flow rate 1ml/minute) into the column. The elution was fractioned at 2ml each. The fractions eluting at the elution volumes of more or less 61ml were each fraction recovered. The protein contained in each of the fractions was analyzed with a 10%-20% SDS-PAGE.

Instead of the crude cell extract in the reaction solution described in Example 5(2), the recovered fractions were added and maintained in the presence of component A, component B, component C and compound (II) labeled with ^{14}C, similarly to Example

5(2). The reaction solutions after the maintenance were TLC analyzed to examine the intensity of the spots corresponding to compound (III) labeled with ^{14}C. The protein moving to the position to 47kDa in the above SDS-PAGE was observed to have its fluctuations in the concentrations of the bands of the fractions added in turn to be parallel with the fluctuations of the intensity of the spots corresponding to compound (III). Said protein was recovered from the SDS-PAGE gel and was subjected to an amino acid sequence analysis with a protein sequencer (Applied Biosystems Company, Procise 494HT, pulsed liquid method) to sequence the N terminus amino acid sequence. As a result, the amino acid sequence shown in SEQ ID NO: 20 was provided. Further, after digesting the above protein with trypsin, the obtained digestion material was analyzed on a mass spectrometer (ThermoQuest Company, Ion Trap Mass Spectrometer LCQ, column: LC Packings Company PepMap C18 75μm x 150mm, solvent A: 0.1% HOAc-H_2O, solvent B: 0.1% HOAc-methanol, gradient: a linear gradient starting at an elution with a mixture of 95% of solvent A and 5% of solvent B and increasing to a concentration of 100% of solvent B over 30 minutes, flow rate: 0.2μl/minute). As a result, the sequence shown in SEQ ID NO: 21 was provided.

Example 6 Obtaining the present invention DNA (A2)

(1) Preparation of the chromosomal DNA of Saccharopolyspora taberi JCM 9383t

Saccharopolyspora taberi JCM 9383t was shake cultured at 30°C for 1 day to 3 days in 50ml of YEME medium (0.3%(w/v) yeast extract, 0.5%(w/v) bacto-peptone, 0.3%(w/v) malt extract, 1.0%(w/v) glucose, 34%(w/v) sucrose and 0.2%(v/v) 2.5M $MgCl_2 \cdot 6H_2O$). The cells were recovered. The obtained cells were suspended in YEME medium containing 1.4%(w/v) glycine and 60mM EDTA and further incubated with

shaking for a day. The cells were recovered from the culture medium. After washing once with distilled water, it was resuspended in buffer (100mM Tris-HCl (pH8.0), 100mM EDTA, 10mM NaCl) at 1ml per 200mg of the cell pellets. Two hundred micrograms per milliliter (200μg/ml) of egg-white lysozyme were added. The cell suspension was shaken at 30℃ for a hour. Further, 0.5% of SDS and 1mg/ml of Proteinase K was added. The cell suspension was incubated at 55℃ for 3 hours. The cell suspension was extracted twice with phenol·chloroform·isoamyl alcohol to recover each of the aqueous layers. Next, there was one extraction with chloroform·isoamyl alcohol to recover the aqueous layer. The chromosomal DNA was obtained by ethanol precipitating the aqueous layer.

(2) Preparation of the chromosomal DNA library of Saccharopolyspora taberi JCM 9383t

Nineteen micrograms (19μg) of the chromosomal DNA prepared in Example 5(1) were digested with 0.78U of restriction enzyme Sau3AI at 37℃ for 60 minutes. The obtained digestion solution was separated with 1% agarose gel electrophoresis. The DNA of about 2.0kbp was recovered from the gel. The DNA was purified with QIAquick Gel Extraction Kit (Qiagen Company) according to the instructions attached to said kit and was concentrated with an ethanol precipitation to obtain 10μl of the solution containing the target DNA. Eight microliters (8μl) of the DNA solution, 100ng of plasmid vector pUC118 digested with restriction enzyme BamHI and treated with dephosphorylation and 12μl of the I solution from Ligation Kit Ver. 2 (Takara Shuzo Company) were mixed and maintained for 3 hours at 16℃. E coli DH5 α was transformed with the ligation solution. The E. coli transformants were cultured overnight at 37℃ in LB agar medium containing 50mg/l of ampicillin. The obtained colonies were

recovered from an agar medium. The plasmids were extracted and were designated as the chromosomal DNA library.

(3) **Isolation of the present invention DNA (A2)**

PCR was conducted by utilizing the chromosomal DNA prepared in Example 6(1) as the template with Expand HiFi PCR System (Boehringer Manheim Company) (Fig. 2). As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 54 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 55 (hereinafter referred to as "primer paring 6"). The nucleotide sequence shown in SEQ ID NO: 54 was designed based on a nucleotide sequence encoding the N terminus amino acid sequence shown in SEQ ID NO: 20. Further, the nucleotide sequence shown in SEQ ID NO: 55 was designed based on a nucleotide sequence complimentary to the nucleotide sequence encoding the inner amino acid sequence shown in SEQ ID NO: 21. The PCR reaction solution amounted to 25µl by adding 300ng of the above chromosomal DNA, the 2 primers each amounting to 7.5pmol, 0.2µl of dNTP mix (a mixture of 2mM of each of the 4 types of dNTP), 2.5µl of 10x buffer (containing $MgCl_2$), 0.19µl of Expand HiFi enzyme mix and distilled water. The reaction conditions of the PCR were after maintaining 97℃ for 2 minutes, repeating 10 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 65℃ for 30 seconds and followed by 72℃ for 1 minute; then conducting 15 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 65℃ for 30 seconds and followed by 72℃ for 1 minute (wherein 20 seconds was added to the maintenance at 72℃ for each cycle); and then maintaining 72℃ for 7 minutes. After the maintenance, the reaction solution was subjected to 2% agarose gel electrophoresis. The gel area containing the DNA of about 800bp was recovered. The DNA was purified from the recovered gel by

utilizing Qiagen quick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA was ligated to the TA cloning vector pCRII-TOPO (Invitrogen Company) according to the instructions attached to said vector and was introduced into E. Coli TOP10F'. The plasmid DNA was prepared from the obtained E. coli transformant, utilizing Qiagen Tip20 (Qiagen Company). A sequencing reaction was conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the -21M13 primer (Applied Biosystems Japan Company) and M13Rev primer (Applied Biosystems Japan Company). The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). As a result, the nucleotide sequence shown in nucleotides 36 to 819 of the nucleotide sequence shown in SEQ ID NO: 10 was provided. Nucleotides 37-60 of the nucleotide sequence shown in SEQ ID NO: 10 encoded a part of the amino acid sequence shown in SEQ ID NO: 20. In this regard, it was expected that that said DNA encoded a part of the present invention protein (A2).

Next, PCR was conducted by utilizing the chromosomal DNA prepared in Example 6(2) as the template and similar to the above with Expand HiFi PCR system. There was utilized as primers, a pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 56 with an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 57 (hereinafter referred to as the "primer pairing 7"). By conducting the PCR with such primers, there was amplified a DNA having a nucleotide sequence in which the 5' terminus elongates past the nucleotide shown as nucleotide 36 of the nucleotide sequence shown in SEQ ID NO: 10. Further, there was utilized as primers, a pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 58 with an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 59 (hereinafter referred to as the "primer pairing 8"). By conducting the PCR with such

primers, there was amplified a DNA having a nucleotide sequence in which the 3' terminus elongates past the nucleotide shown as nucleotide 819 of the nucleotide sequence shown in SEQ ID NO: 10. Each of the 1.3kb DNA amplified with the use of primer pairing 7 and the 0.4kb DNA amplified with the use of primer pairing 8 was cloned into TA cloning vector pCRII-TOPO. Plasmid DNA was prepared from the obtained E. coli transformant, utilizing Qiagen Tip 20 (Qiagen Company). A sequencing reaction was conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the -21M13 primer (Applied Biosystems Japan Company), M13Rev primer (Applied Biosystems Japan Company) and the oligonucleotide shown in SEQ ID NO: 60. The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). As a result of sequencing the nucleotide sequence of the 1.3kb DNA amplified by utilizing primer pairing 7, the nucleotide sequence shown in nucleotides 1 to 35 of the nucleotide sequence shown in SEQ ID NO: 10 was provided. Further, as a result of sequencing the nucleotide sequence of the 0.4kb DNA amplified by utilizing primer pairing 8, the nucleotide sequence shown in nucleotides 819 to 1415 of the nucleotide sequence shown in SEQ ID NO: 10 was provided. As a result of connecting the obtained nucleotide sequences, the nucleotide sequence shown in SEQ ID NO: 10 was obtained. Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 7) consisting of 1206 nucleotides (inclusive of the stop codon) and encoding a 401 amino acid residue as well as a nucleotide sequence (SEQ ID NO: 16) consisting of 198 nucleotides (inclusive of the stop codon) and encoding a 65 amino acid residue. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 2) encoded by the nucleotide sequence shown in SEQ ID NO: 7 was calculated to be 43983Da. Further, the amino acid sequence

encoded by said nucleotide sequence contained the amino acid sequence (SEQ ID NO: 20) determined from the amino acid sequencing of from the N terminus of the present invention protein (A2) and the amino acid sequence (SEQ ID NO: 21) determined from the amino acid sequencing of the mass spectrometer analysis with the trypsin digestion fragments. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 13) encoded by the nucleotide sequence shown in SEQ ID NO: 16 was calculated be 6707Da.

Example 7 Expression of the Present Invention Protein (A2) in E. coli

(1) Production of a transformed E. coli having the present invention protein (A2)

PCR was conducted by utilizing as a template the chromosomal DNA prepared from Saccharopolyspora taberi JCM 9383t in Example 6(1) and by utilizing Expand HiFi PCR System (Boehringer Manheim Company). As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 61 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 62 (hereinafter referred to as "primer pairing 21") or a pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 61 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 63 (hereinafter referred to as "primer pairing 22"). The PCR reaction solution amounted to 50µl by adding the 2 primers each amounting to 300nM, 50ng of the above chromosomal DNA, 5.0µl of dNTP mix (a mixture of 2.0mM of each of the 4 types of dNTP), 5.0µl of 10x Expand HF buffer (containing $MgCl_2$) and 0.75µl of Expand HiFi enzyme mix and distilled water. The reaction conditions of the PCR were after maintaining 97°C for 2 minutes; repeating 10 cycles of a cycle that included maintaining 97°C for 15 seconds, followed by 60°C for 30 seconds and followed by 72°C for 1 minute; then conducting 15 cycles of a cycle that included maintaining 97°C for 15 seconds, followed by 60°C for 30 seconds and followed

by 72℃ for 1 minute (wherein 20 seconds was added to the maintenance at 72℃ for each cycle); and then maintaining 72℃ for 7 minutes. After the maintenance, the reaction solution was subjected to 1% agarose gel electrophoresis. The gel area containing the DNA of about 1.2kbp was recovered from the gel which was subjected the reaction solution utilizing primer pairing 21. The gel area containing the DNA of about 1.4kbp was recovered from the gel which was subjected the reaction solution utilizing primer pairing 22. The DNA were purified from each of the recovered gels by utilizing Qiagen quick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA were ligated to the cloning vector pCRII-TOPO (Invitrogen Company) according to the instructions attached to said vector and were introduced into E. Coli TOP10F'. The plasmid DNA were prepared from the obtained E. coli transformants, utilizing Qiagen Tip20 (Qiagen Company). Next, sequencing reactions were conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the -21M13 primer (Applied Biosystems Japan Company), M13Rev primer (Applied Biosystems Japan Company), the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 56 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 64. The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). Based on the results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 7 was designated as pCR923 and the plasmid having the nucleotide sequence shown in SEQ ID NO: 10 was designated as pCR923F.

Next, each of plasmids pCR923 and pCR923F was digested with restriction enzymes NdeI and HindIII. The digestion products were subjected to agarose gel electrophoresis. The gel area containing a DNA of about 1.2kbp was cut from the gel subjected to the digestion products of pCR923. The gel area containing a DNA of about

1.4kbp was cut from the gel subjected to the digestion products of pCR923F. The DNA were purified from each of the recovered gels by utilizing Qiagen quick gel extraction kit (Qiagen Company) according to the attached instructions. Each of the obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated with ligation kit Ver.1 (Takara Shuzo Company) according to the instructions attached to said kit and introduced into E. Coli JM109. The plasmid DNA were prepared from the obtained E. coli transformants. The structures thereof were analyzed. The plasmid containing the nucleotide sequence shown in SEQ ID NO: 7, in which the DNA of about 1.2kbp encoding the present invention protein (A2) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN923. Further, the plasmid containing the nucleotide sequence shown in SEQ ID NO: 10, in which the DNA of about 1.4kbp encoding the present invention protein (A2) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN923F. Each of the above plasmids of pKSN923 and pKSN923F was introduced into E. coli JM109. The obtained E. coli transformants were designated, respectively, JM109/pKSN923 and JM109/pKSN923F. Further, plasmid pKSN2 was introduced into E. coli JM109. The obtained E. coli transformant was designated as JM109/pKSN2.

(2) Expression of the present invention protein (A2) in E. coli and recovery of said protein

E. coli JM109/pKSN657, JM109/pKSN657F and JM109/pKSN2 were each cultured overnight at 37℃ in 10ml of TB medium (1.2%(w/v) tryptone, 2.4%(w/v) yeast extract, 0.4%(w/v) glycerol, 17mM potassium dihydrogenphosphate, 72mM dipotassium hydrogenphosphate) containing 50μg/ml of ampicillin. A milliliter (1ml) of the obtained culture medium was transferred to 100ml of TB medium containing 50μg/ml of

ampicillin and cultured at 26℃. When OD660 reached about 0.5, 5-aminolevulinic acid was added to the final concentration of 500μM, and the culturing was continued. Thrity (30) minutes thereafter, IPTG was added to a final concentration of 1mM, and there was further culturing for 17 hours.

The cells were recovered from each of the culture mediums, washed with 0.1M tris-HCl buffer (pH7.5) and suspended in 10 ml of said buffer containing 1mM PMSF. The obtained cell suspensions were subjected 6 times to a sonicator (Sonifier (Branson Sonic Power Company)) at 3 minutes each under the conditions of output 3, duty cycle 30%, in order to obtain cell lysate solutions. After centrifuging the cell lysate solutions (1,200xg, 5 minutes) the supernatants were recovered and centrifuged (150,000xg, 70 minutes) to recover supernatant fractions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN923 is referred to as "E. coli pKSN923 extract ", the supernatant fraction obtained from E. coli JM109/pKSN923F is referred to as "E. coli pKSN923F extract", and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract "). A microliter (1μl) of the above supernatant fractions was analyzed on a 15% to 25% SDS-PAGE and stained with CBB. As a result, notably more intense bands were detected in both E. coli pKSN923 extract and E. coli pKSN923F extract than the E. coli pKSN2 extract, at the electrophoresis locations corresponding to the molecular weight of 47kDa. It was confirmed that E. coli JM109/pKSN923 and E. coli JM109/pKSN923F expressed the present invention protein (A2).

(3) Detection of the ability to convert compound (II) to compound (III)

Reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. The reaction solutions consisted of a 0.1M potassium phosphate buffer (pH7.0)

containing 3ppm of compound (II) labeled with ^{14}C, 2mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 0.2mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 18μl of the supernatant fraction recovered in Example 7(2). Further, there were prepared and maintained similarly reaction solutions having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B and component C. Three microliters (3μl) of 2N HCl and 90 μl of ethyl acetate were added and mixed into each of the reaction solutions after the maintenance. The resulting reaction solutions were centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof was spotted to a silica gel TLC plate (TLC plate silica gel $60F_{254}$, 20cm x 20cm, 0.25mm thick, Merck Company). The TLC plate was developed with a 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents were then allowed to evaporate. The TLC plate was exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). The results are shown in Table 8.

Table 8

Reaction components					spot of compound (III)
component A	component B	component C	E. coli extract	compound (II) labeled with ^{14}C	
+	+	+	−	+	−
+	+	+	pKSN2	+	−
+	+	+	pKSN923	+	+
−	+	+	pKSN923	+	−
+	−	+	pKSN923	+	−
+	+	−	pKSN923	+	+
+	+	+	pKSN923F	+	+
−	+	+	pKSN923F	+	−
+	−	+	pKSN923F	+	−
+	+	−	pKSN923F	+	+

Example 8 Preparation of the Present Protein (A10)

(1) Preparation of the crude cell extract

A frozen stock of Streptomyces griseolus ATCC 11796 was added to 250ml of B medium (1%(w/v) glucose, 0.1%(w/v) meat extract, 0.2%(w/v) tryptose) in a 500ml baffled flask and incubated with rotary shaking at 30℃ for 3 days to obtain a pre-culture. Forty milliliters (40ml) of the pre-culture was added to 400ml of B medium and was incubated with rotary shaking in a 1L triangular flask at 30℃ for 24 hours. After stopping the culturing, the culture was allowed to settle. Two hundred and twenty milliliters (220ml) of only the supernatant was removed. Two hundred and twenty milliliters (220ml) of fresh medium similarly prepared was added to the remaining 220ml of the culture medium to amount to 440ml. Compound (II) was added thereto to amount to 100ppm. The cells were incubated with rotary shaking in the 1L triangular flask at 30℃ for 40 hours. Cell pellets were recovered by centrifuging (3,000g, 5 min.) 2.6L of

the resulting culture. The resulting cell pellets were washed with 1L of 0.1M PIPES-NaOH buffer (pH6.8) to provide 26g of the cell pellets.

These cell pellets were suspended of 0.1M PIPES-NaOH buffer (pH6.8) at 3ml for 1g of the cell pellets, and 1mM of PMSF, 5mM of benzamidine HCl, 1mM of EDTA, 3μg/ml of leupeptin, 3μg/ml of pepstatin A and 1mM of dithiotritol were added. A cell lysate solution was obtained by disrupting twice repetitively the suspension with a French press (1000kg/cm^2) (Ohtake Seisakusho) . After centrifuging the cell lysate solution (40,000xg, 30 minutes), the supernatant was recovered and centrifuged for 1 hour at 150,000xg to recover the supernatant (hereinafter referred to as the "crude cell extract").

(2) Determination of the ability of converting compound (II) to compound (III)

There was prepared 30μl of a reaction solution of 0.1M potassium phosphate buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2.4mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 0.5mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 18μl of the crude cell extract recovered in Example 8(1). The reaction solution was maintained at 30℃ for a hour. Further, there was prepared and maintained similarly a reaction solution having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B and component C. Three microliters (3μl) of 2N HCl and 90 μl of ethyl acetate were added and stirred into each of the reaction solutions after the maintenance. The resulting reaction solutions were centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof was spotted to a

silica gel TLC plate (TLC plate silica gel $60F_{254}$, 20cm x 20cm, 0.25 thick, Merck Company). The TLC plate was developed with a 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents were then allowed to evaporate. The TLC plate was exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). The results are shown in Table 9.

Table 9

Reaction components					spot of compound (III)
component A	component B	component C	crude cell extract	compound (II) labeled with ^{14}C	
+	+	+	−	+	−
+	+	+	+	+	+
−	+	+	+	+	−
+	−	−	+	+	−

(3) **Fractionation of the crude cell extract**

Ammonium sulfate was added to the crude cell extract obtained in Example 8(1) to amount to 45% saturation. After stirring in ice-cooled conditions, the supernatant was recovered by centrifuging for 10 minutes at 12,000xg. After adding ammonium sulfate to the obtained supernatant to amount to 55% saturation and stirring in ice-cooled conditions, a pellet was recovered by centrifuging for 10 minutes at 12,000xg. The pellet was dissolved with 20mM bistrispropane buffer (pH7.0) to amount to 10ml. This solution was subjected to a PD10 column (Amersham Pharmacia Company) and eluted with 20mM of bistrispropane buffer (pH7.0) to recover 14ml of fractions containing proteins (hereinafter referred to as the "45-55% ammonium sulfate fraction").

(4) **Isolation of the present protein (A10)**

The 45-55% ammonium sulfate fraction prepared in Example 8(3) was injected into a MonoQ HR 10/10 column (Amersham Pharmacia Company). Next, after flowing 16ml of 20mM bistrispropane buffer (pH7.0) into the column, 20mM bistrispropane buffer was flown with a linear gradient of NaCl (gradient of NaCl was 0.00625M/minute, range of NaCl concentration was from 0M to 0.5M, flow rate was 4ml/minute) to fraction recover 15ml of fractions eluting at the NaCl concentration of from 0.28M to 0.31M. Further, the recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) and eluted with 20mM bistrispropane buffer (pH7.0) to recover the fractions containing protein.

The recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) with the elution with Buffer A (2mM potassium phosphate buffer containing 1.5mM of NaCl, pH 7.0), in order to recover the fractions containing protein. Next, the fractions were injected into a Bio-Scale Ceramic Hydroxyapatite Type I column CHT10-I (BioRad Company). Fifty milliliters (50ml) of Buffer A was flown into the column. Subsequently, Buffer A was flown with a linear gradient of Buffer B (100mM potassium phosphate buffer containing 0.03mM of NaCl; the linear gradient started at 100% Buffer A to increase to 50% Buffer B over a 40 minute period, flow rate was 5ml/minute) to fraction recover the fractions eluting at a Buffer B concentration of from 16% to 31%. Further, the recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) and eluted with 0.05M potassium phosphate buffer (pH7.0) to recover the fractions containing protein. The protein contained in each of the fractions were analyzed on a 10%-20% SDS-PAGE.

Instead of the crude cell extract in the reaction solution described in Example 8(2), the recovered fractions were added and maintained in the presence of component A,

component B, component C and compound (II) labeled with ^{14}C, similarly to Example 8(2). The reaction solutions after the maintenance were TLC analyzed to examine the intensity of the spots corresponding to compound (III) labeled with ^{14}C. The protein moving to the position to 47kDa in the above SDS-PAGE was observed to have its fluctuations in the concentrations of the bands of the fractions added in turn to be parallel with the fluctuations of the intensity of the spots corresponding to compound (III). Said protein was recovered from the SDS-PAGE gel and digested with trypsin. The obtained digestion material was analyzed on a mass spectrometer (ThermoQuest Company, Ion Trap Mass Spectrometer LCQ, column: LC Packings Company PepMap C18 75μm x 150mm, solvent A: 0.1%HOAc-H_2O, solvent B: 0.1% HOAc-methanol, gradient: a linear gradient starting at an elution with a mixture of 95% of solvent A and 5% of solvent B and increasing to a concentration of 100% of solvent B over 30 minutes, flow rate: 0.2μl/minute). As a result, the amino acid sequences shown in each and any one of SEQ ID NO: 22-34 were provided.

Example 9 Preparation of the Chromosomal DNA of Streptomyces Griseolus ATCC 11796

Streptomyces griseolus ATCC 11796 was incubated with shaking at 30℃ for 1 day to 3 days in 50ml of YEME medium (0.3%(w/v) yeast extract, 0.5%(w/v) bacto-peptone, 0.3%(w/v) malt extract, 1.0%(w/v) glucose, 34%(w/v) sucrose and 0.2%(v/v) 2.5M $MgCl_2 \cdot 6H_2O$). The cells were recovered. The obtained cells were suspended in YEME medium containing 1.4%(w/v) glycine and 60mM EDTA and further incubated with shaking for a day. The cells were recovered from the culture medium. After washing once with distilled water, it was resuspended in buffer (100mM Tris-HCl (pH8.0), 100mM EDTA, 10mM NaCl) at 1ml per 200mg of the cells. Two hundred

micrograms per milliliter (200μg/ml) of egg-white lysozyme were added. The cell suspension was shaken at 30℃ for a hour. Further, 0.5% of SDS and 1mg/ml of Proteinase K was added. The cell suspension was incubated at 55℃ for 3 hours. The cell suspension was extracted twice with phenol·chloroform·isoamyl alcohol to recover each of the aqueous layers. Next, there was one extraction with chloroform·isoamyl alcohol to recover the aqueous layer. The chromosomal DNA was obtained by ethanol precipitating the aqueous layer.

Example 10 Obtaining a DNA Encoding the Present DNA (A10) and Expression in E. coli

(1) Production of a transformed E. coli having the present DNA

PCR was conducted by utilizing as a template the chromosomal DNA prepared from Streptomyces griseolus ATCC 11796 in Example 9 and by utilizing Expand High Fidelity PCR System (Roche Molecular Biochemicals Company). As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 79 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 80 (hereinafter referred to as "primer pairing 23") or a pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 79 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 81 (hereinafter referred to as "primer pairing 24"). The PCR reaction solutions amounted to 50μl by adding the 2 primers each amounting to 300nM, 50ng of the above chromosomal DNA, 5.0μl of dNTP mix (a mixture of 2.0mM of each of the 4 types of dNTP), 5.0μl of 10x Expand HF buffer (containing $MgCl_2$) and 0.75μl of Expand HiFi enzyme mix and distilled water. The reaction conditions of the PCR were after maintaining 97℃ for 2 minutes; repeating 10 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 65℃ for 30

seconds and followed by 72℃ for 2 minutes; then conducting 15 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 68℃ for 30 seconds and followed by 72℃ for 2 minutes (wherein 20 seconds was added to the maintenance at 72℃ for each cycle); and then maintaining 72℃ for 7 minutes. After the maintenance, each of the reaction solutions was subjected to 1% agarose gel electrophoresis. The gel area containing the DNA of about 1.2kbp was recovered from the gel which was subjected the reaction solution utilizing primer pairing 23. The gel area containing the DNA of about 1.5kbp was recovered from the gel which was subjected the reaction solution utilizing primer pairing 24. The DNA were purified from each of the recovered gels by utilizing Qiagen quick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA were ligated to the cloning vector pCR2.1-TOPO (Invitrogen Company) according to the instructions attached to said vector and were introduced into E. Coli TOP10F'. The plasmid DNA were prepared from the obtained E. coli transformants, utilizing Qiaprep Spin Miniprep Kit (Qiagen Company). Next, sequencing reactions were conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the -21M13 primer (Applied Biosystems Japan Company), M13Rev primer (Applied Biosystems Japan Company), the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 82 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 83. The sequencing reactions utilized the obtained plasmid DNA as the template. The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). Based on the results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 84 was designated as pCR11796 and the plasmid having the nucleotide sequence shown in SEQ ID NO: 85 was designated as pCR11796F. Two open reading frames (ORF) were present in said nucleotide sequence

shown in SEQ ID NO: 85. As such, there was contained a nucleotide sequence (SEQ ID NO: 84) consisting of 1221 nucleotides (inclusive of the stop codon) and encoding a 406 amino acid residue (the amino acid sequence shown in SEQ ID NO: 5) and a nucleotide sequence consisting of 210 nucleotides (inclusive of the stop codon) and encoding a 69 amino acid residue.

Next, each of pCR11796 and pCR11796F was digested with restriction enzymes NdeI and HindIII. The digestion products were subjected to agarose gel electrophoresis. The gel area containing a DNA of about 1.2kbp was cut from the gel subjected to the digestion products of pCR11796. The gel area containing a DNA of about 1.5kbp was cut from the gel subjected to the digestion products of pCR11796F. The DNA were purified from each of the recovered gels by utilizing Qiagen quick gel extraction kit (Qiagen Company) according to the attached instructions. Each of the obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated with ligation kit Ver.1 (Takara Shuzo Company) according to the instructions attached to said kit and introduced into E. Coli JM109. The plasmid DNA were prepared from the obtained E. coli transformants. The structures thereof were analyzed. The plasmid containing the nucleotide sequence shown in SEQ ID NO: 84, in which the DNA of about 1.2kbp encoding the present protein (A10) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN11796. Further, the plasmid containing the nucleotide sequence shown in SEQ ID NO: 85, in which the DNA of about 1.5kbp encoding the present protein (A10) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN11796F. Each of the above plasmids of pKSN11796 and pKSN11796F was introduced into E. coli JM109. The obtained E. coli transformants were designated, respectively, JM109/pKSN11796 and JM109/pKSN11796F. Further, plasmid pKSN2 was introduced into E. coli JM109. The obtained E. coli transformant

was designated as JM109/pKSN2.

(2) **Expression of the present protein (A10) in E. coli and recovery of said protein**

E. coli JM109/pKSN11796, JM109/pKSN11796F and JM109/pKSN2were each cultured overnight at 37℃ in 10ml of TB medium (1.2%(w/v) tryptone, 2.4%(w/v) yeast extract, 0.4%(w/v) glycerol, 17mM potassium dihydrogenphosphate, 72mM dipotassium hydrogenphosphate) containing 50μg/ml of ampicillin. A milliliter (1ml) of the obtained culture medium was transferred to 100ml of TB medium containing 50μg/ml of ampicillin and cultured at 26℃. When OD660 reached about 0.5, 5-aminolevulinic acid was added to the final concentration of 500μM, and the culturing was continued. Thirty (30) minutes thereafter, IPTG was added to a final concentration of 1mM, and there was further culturing for 17 hours.

The cells were recovered from each of the culture mediums, washed with 0.1M tris-HCl buffer (pH7.5) and suspended in 10ml of the above buffer containing 1mM PMSF. The obtained cell suspensions were subjected 6 times to a sonicator (Sonifier (Branson Sonic Power Company)) at 3 minutes each under the conditions of output 3, duty cycle 30%, in order to obtain cell lysate solutions. After centrifuging the cell lysate solutions (1,200xg, 5 minutes) the supernatants were recovered and centrifuged (150,000xg, 70 minutes) to recover supernatant fractions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN11796 is referred to as "E. coli pKSN11796 extract ", the supernatant fraction obtained from E. coli JM109/pKSN11796F is referred to as "E. coli pKSN11796F extract", and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract "). A microliter (1μl) of the above supernatant fractions was analyzed on a 15% to 25% SDS-PAGE and stained with

Coomasie Blue (hereinafter referred to as "CBB"). As a result, notably more intense bands were identified in both E. coli pKSN11796 extract and E. coli pKSN11796F extract than the E. coli pKSN2 extract, at the electrophoresis locations corresponding to the molecular weight of 45kDa. A more intense band was identified in E. coli pKSN11796F extract than E. coli pKSN11796 extract. It was shown that E. coli JM109/pKSN11796F expressed the present protein (A10) to a higher degree than E. coli JM109/pKSN11796.

(3) Detection of the ability to convert compound (II) to compound (III)

Reaction solutions of 30μl were prepared and maintained for 1 hour at 30℃. The reaction solutions consisted of a 0.1M potassium phosphate buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 2mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 0.1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 18μl of the supernatant fraction recovered in Example 10(2). Further, there were prepared and maintained similarly reaction solutions having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B and component C. Three microliters (3μl) of 2N HCl and 90μl of ethyl acetate were added and mixed into each of the reaction solutions after the maintenance. The resulting reaction solutions were centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof was spotted to a silica gel TLC plate (TLC plate silica gel $60F_{254}$ 20cm x 20cm, 0.25mm thick, Merck Company). The TLC plate was developed with a 6: 1: 2 mixture of chloroform, acetic

acid and ethyl acetate for about 1 hour. The solvents were then allowed to evaporate. The TLC plate was exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). The results are shown in Table 10.

Table 10

Reaction components					spot of compound (III)
component A	component B	component C	E. coli extract	compound (II) labeled with ^{14}C	
+	+	+	−	+	−
+	+	+	pKSN2	+	−
+	+	+	pKSN11796	+	+
−	+	+	pKSN11796	+	−
+	−	+	pKSN11796	+	−
+	+	−	pKSN11796	+	+
+	+	+	pKSN11796F	+	+
−	+	+	pKSN11796F	+	−
+	−	+	pKSN11796F	+	−
+	+	−	pKSN11796F	+	+

Example 11 Obtaining the Present Invention DNA (A3)

(1) Preparation of the Chromosomal DNA of Streptomyces testaceus ATCC21469

Streptomyces testaceus ATCC21469 was incubated with shaking at 30℃ for 1 day to 3 days in 50ml of YEME medium (0.3%(w/v) yeast extract, 0.5%(w/v) bacto-peptone, 0.3%(w/v) malt extract, 1.0%(w/v) glucose, 34%(w/v) sucrose and 0.2%(v/v) 2.5M $MgCl_2 \cdot 6H_2O$). The cells were recovered. The obtained cells were suspended in YEME medium containing 1.4%(w/v) glycine and 60mM EDTA and further incubated

with shaking for a day. The cells were recovered from the culture medium. After washing once with distilled water, it was resuspended in buffer (100mM Tris-HCl (pH8.0), 100mM EDTA, 10mM NaCl) at 1ml per 200mg of the cells. Two hundred micrograms per milliliter (200μg/ml) of egg-white lysozyme were added. The cell suspension was shaken at 30℃ for a hour. Further, 0.5% of SDS and 1mg/ml of Proteinase K was added. The cell suspension was incubated at 55℃ for 3 hours. The cell suspension was extracted twice with phenol·chloroform·isoamyl alcohol to recover each of the aqueous layers. Next, there was one extraction with chloroform·isoamyl alcohol to recover the aqueous layer. The chromosomal DNA was obtained by ethanol precipitating the aqueous layer.

(2) Isolation of the present invention DNA (A3)

PCR was conducted by utilizing the chromosomal DNA prepared in Example 11(1) as the template. As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 65 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 66 (hereinafter referred to as "primer pairing 9"). The PCR reaction solution amounted to 50μl by adding 250ng of the above chromosomal DNA, the 2 primers each amounting to 200nM, 4μl of dNTP mix (a mixture of 2.5mM of each of the 4 types of dNTP), 5μl of 10x ExTaq buffer, 0.5μl of ExTaq polymerase (Takara Shuzo Company) and distilled water. The reaction conditions of the PCR were maintaining 97℃ for 2 minutes; repeating 30 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 90 seconds; and then maintaining 72℃ for 4 minutes. After the maintenance, the reaction solution was subjected to 0.8% agarose gel electrophoresis. The gel area containing the DNA of about 1.4kbp was recovered. The DNA was purified from the

recovered gel by utilizing QIAquick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA was ligated to the TA cloning vector pCR2.1 (Invitrogen Company) according to the instructions attached to said vector and was introduced into E. Coli TOP10F'. The plasmid DNA was prepared from the obtained E. coli transformant, utilizing QIAprep Spin Miniprep Kit (Qiagen Company). A sequencing reaction was conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 67 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 68. The sequencing reactions utilized the obtained plasmid as the template. The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). As a result, the nucleotide sequence shown in SEQ ID NO: 69 was provided. Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence consisting of 1188 nucleotides (inclusive of the stop codon) and encoding a 395 amino acid residue and a nucleotide sequence (SEQ ID NO: 17) consisting of 195 nucleotides (inclusive of the stop codon) and encoding a 64 amino acid residue. The molecular weight of the amino acid sequence encoded by the nucleotide sequence shown in SEQ ID NO: 17 was calculated to be 6666Da.

Example 12 Expression of the Present Invention Protein (A3) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A3)

PCR was conducted by utilizing as a template the chromosomal DNA prepared in Example 11(1) and by utilizing ExTaq polymerase (Takara Shuzo Company) under similar conditions as above. As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 70 and an

oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 71 (hereinafter referred to as "primer pairing 10") or a pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 70 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 72 (hereinafter referred to as "primer pairing 11"). The DNA of 1.2kb amplified by utilizing the primer pairing 10 and the DNA of 1.5kbp amplified by utilizing the primer pairing 11 were cloned into TA cloning vector pCR2.1 according to the above methods. The plasmid DNA were prepared from the obtained E. coli transformants, utilizing QIAprep Spin Miniprep Kit (Qiagen Company). Sequencing reactions were conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 67 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 68. The sequencing reactions utilized the obtained plasmid DNA as the template. The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). As a result, the plasmid cloned with the DNA amplified by the primer pairing 10 was confirmed to have the nucleotide sequence shown in SEQ ID NO: 8. The plasmid cloned with the DNA amplified by primer pairing 11 was confirmed to have the nucleotide sequence shown in SEQ ID NO: 11. Two open reading frames (ORF) were present in said nucleotide sequence shown in SEQ ID NO: 11. As such, there was contained a nucleotide sequence (SEQ ID NO: 8) consisting of 1188 nucleotides (inclusive of the stop codon) and encoding a 395 amino acid residue and a nucleotide sequence consisting of 195 nucleotides (inclusive of the stop codon) and encoding a 64 amino acid residue. The molecular weight of the protein consisting of the amino acid sequence encoded by the nucleotide sequence shown in SEQ ID NO: 8 was calculated to be 43752Da. With the obtained plasmids, the plasmid having the nucleotide sequence shown in SEQ ID NO: 8 was

designated as pCR671 and the plasmid having the nucleotide sequence shown in SEQ ID NO: 11 was designated as pCR671F.

Next, each of pCR671 and pCR671F was digested with restriction enzymes NdeI and HindIII. The digestion products were subjected to agarose gel electrophoresis. The gel area containing a DNA of about 1.2kbp was cut from the gel subjected to the digestion products of pCR671. The gel area containing a DNA of about 1.5kbp was cut from the gel subjected to the digestion products of pCR671F. The DNA were purified from each of the recovered gels by utilizing Qiagen quick gel extraction kit (Qiagen Company) according to the attached instructions. Each of the obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated with ligation kit Ver.1 (Takara Shuzo Company) according to the instructions attached to said kit and introduced into E. Coli JM109. The plasmid DNA were prepared from the obtained E. coli transformants. The structures thereof were analyzed. The plasmid containing the nucleotide sequence shown in SEQ ID NO: 8, in which the DNA of about 1200bp encoding the present invention protein (A3) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN671. Further, the plasmid containing the nucleotide sequence shown in SEQ ID NO: 11, in which the DNA of about 1400bp encoding the present invention protein (A3) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN671F. Each of the above plasmids of pKSN671 and pKSN671F was introduced into E. coli JM109. The obtained E. coli transformants were designated, respectively, JM109/pKSN671 and JM109/pKSN671F. Further, plasmid pKSN2 was introduced into E. coli JM109. The obtained E. coli transformant was designated as JM109/pKSN2.

(2) Expression of the present invention protein (A3) in E. coli and recovery of

said protein

E. coli JM109/pKSN671, JM109/pKSN671F and JM109/pKSN2 were each cultured overnight at 37℃ in 10ml of TB medium (1.2%(w/v) tryptone, 2.4%(w/v) yeast extract, 0.4%(w/v) glycerol, 17mM potassium dihydrogenphosphate, 72mM dipotassium hydrogenphosphate) containing 50μg/ml of ampicillin. A milliliter (1ml) of the obtained culture medium was transferred to 100ml of TB medium containing 50μg/ml of ampicillin and cultured at 26℃. When OD660 reached about 0.5, 5-aminolevulinic acid was added to the final concentration of 500μM, and the culturing was continued. Thirty (30) minutes thereafter, IPTG was added to a final concentration of 1mM, and there was further culturing for 17 hours.

The cells were recovered from each of the culture mediums, washed with 0.1M tris-HCl buffer (pH7.5) and suspended in 10ml of said buffer containing 1mM PMSF. The obtained cell suspensions were subjected 6 times to a sonicator (Sonifier (Branson Sonic Power Company)) at 3 minutes each under the conditions of output 3, duty cycle 30%, in order to obtain cell lysate solutions. After centrifuging the cell lysate solutions (1,200xg, 5 minutes) the supernatants were recovered and centrifuged (150,000xg, 70 minutes) to recover supernatant fractions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN671 is referred to as "E. coli pKSN671 extract ", the supernatant fraction obtained from E. coli JM109/pKSN671F is referred to as "E. coli pKSN671F extract", and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Reaction solutions of 30μl were prepared and maintained for 1 hour at 30℃. The reaction solutions consisted of a 0.1M potassium phosphate buffer (pH7.0) containing

3ppm of compound (II) labeled with ^{14}C, 2mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 2mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 0.1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 18μl of the supernatant fraction recovered in Example 12(2). Further, there were prepared and maintained similarly reaction solutions having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B and component C. Three microliters (3μl) of 2N HCl and 90μl of ethyl acetate were added and stirred into each of the reaction solutions after the maintenance. The resulting reaction solutions were centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof was spotted to a silica gel TLC plate (TLC plate silica gel $60F_{254}$, 20cm x 20cm, 0.25mm thick, Merck Company). The TLC plate was developed with a 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents were then allowed to evaporate. The TLC plate was exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). The results are shown in Table 11.

Table 11

Reaction components					spot of compound (III)
component A	component B	component C	E. coli extract	compound (II) labeled with ^{14}C	
+	+	+	−	+	−
+	+	+	pKSN2	+	−
+	+	+	pKSN671	+	+
−	+	+	pKSN671	+	−
+	−	+	pKSN671	+	−
+	+	−	pKSN671	+	+
+	+	+	pKSN671F	+	+
−	+	+	pKSN671F	+	−
+	−	+	pKSN671F	+	−
+	+	−	pKSN671F	+	+

Example 13 Obtaining the Present DNA (A9)

(1) Preparation of the chromosomal DNA of Streptomyces carbophilus SANK62585

Streptomyces carbophilus SANK62585 (FERM BP-1145) was incubated with shaking at 30℃ for 1 day in 50ml of YEME medium (0.3%(w/v) yeast extract, 0.5%(w/v) bacto-peptone, 0.3%(w/v) malt extract, 1.0%(w/v) glucose, 34%(w/v) sucrose and 0.2%(v/v) 2.5M $MgCl_2 \cdot 6H_2O$). The cells were then recovered. The obtained cells were suspended in YEME medium containing 1.4%(w/v) glycine and 60mM EDTA and further incubated with shaking for a day. The cells were recovered from the culture medium. After washing once with distilled water, it was resuspended in buffer (100mM Tris-HCl (pH8.0), 100mM EDTA, 10mM NaCl) at 1ml per 200mg of the cells. Two hundred micrograms per milliliter (200μg/ml) of egg-white lysozyme were added. The cell suspension was shaken at 30℃ for a hour. Further, 0.5% of SDS and 1mg/ml of

Proteinase K was added. The cell suspension was incubated at 55℃ for 3 hours. The cell suspension was extracted twice with phenol·chloroform·isoamyl alcohol to recover each of the aqueous layers. Next, there was one extraction with chloroform·isoamyl alcohol to recover the aqueous layer. The chromosomal DNA was obtained by ethanol precipitating the aqueous layer.

(2) Isolation of the present DNA (A9)

PCR was conducted by utilizing as the template the chromosomal DNA prepared in Example 13(1). As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 74 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 75 (hereinafter referred to as "primer paring 12") or the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 76 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 77 (hereinafter referred to as "primer paring 13"). The PCR reaction solution amounted to 50μl by adding the 2 primers each amounting to 200nM, 250ng of the above chromosomal DNA, 4μl of dNTP mix (a mixture of 2.5mM of each of the 4 types of dNTP), 5μl of 10x ExTaq buffer, 0.5μl of ExTaq polymerase (Takara Shuzo Company) and distilled water. The reaction conditions of the PCR were maintaining 95℃ for 2 minutes; repeating 30 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds, followed by 72℃ for 90 seconds, and then maintaining 72℃ for 4 minutes. After the maintenance, the reaction solution was subjected to 0.8% agarose gel electrophoresis. The gel area containing the DNA of about 500bp was recovered from the gel subjected to the PCR reaction solution utilizing primer pairing 12. The gel area containing the DNA of about 800bp was recovered from the gel subjected to the PCR reaction solution utilizing primer pairing 13. The DNA were purified from each

of the recovered gels by utilizing QIAquick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA were ligated to the TA cloning vector pCR2.1 (Invitrogen Company) according to the instructions attached to said vector and was introduced into E. Coli TOP10F'. The plasmid DNA were prepared from the obtained E. coli transformants, utilizing QIAprep Spin Miniprep Kit (Qiagen Company). A sequencing reaction was conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO:67 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 68. The sequencing reaction utilized the obtained plasmid DNA as the templates. The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). As a result, the nucleotide sequence shown in nucleotides 1 to 498 of the nucleotide sequence shown in SEQ ID NO: 78 was provided by the DNA obtained by the PCR utilizing primer pairing 12. The nucleotide sequence shown in nucleotides 469 to1233 of the nucleotide sequence shown in SEQ ID NO: 78 was provided by the DNA obtained by the PCR utilizing primer pairing 13. The plasmid having the nucleotide sequence of nucleotides 1 to 498 shown in SEQ ID NO: 78 was designated as pCRSCA1. The plasmid having the nucleotide sequence of nucleotides 469 to 1233 shown in SEQ ID NO: 78 was designated as pCRSCA2.

Example 14 Expression of the Present Protein (A9) in E. Coli

(1) Production of a transformed E. coli having the present DNA (A9)

With the plasmids obtained in Example 13(2), the above plasmid pCRSCA1 was digested with NdeI and NcoI and pCRSCA2 was digested with NdeI and NcoI. The digestion products were subjected to agarose gel electrophoresis. The gel area containing

a DNA of about 500bp was cut from the gel subjected to the digestion products of pCRSCA2. The gel area containing a DNA of about 800bp was cut from the gel subjected to the digestion products of pCRSCA2. The DNA were purified from each of the recovered gels by utilizing QIAquick gel extraction kit (Qiagen Company) according to the attached instructions. The 2 types of the obtained DNA were ligated together with the plasmid pKSN2 digested with NdeI and HindIII, utilizing ligation kit Ver.1 (Takara Shuzo Company) in accordance with the instructions attached to said kit and introduced into E. Coli JM109. The plasmid DNA was prepared from the obtained E. coli transformants. The structure thereof was analyzed. The plasmid containing the nucleotide sequence shown in SEQ ID NO: 78, in which the DNA encoding the present protein (A9) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSNSCA.

(2) Expression of the present protein (A9) in E. coli and recovery of said protein

E. coli JM109/pKSNSCA was cultured overnight at 37℃ in 10ml of TB medium (1.2%(w/v) tryptone, 2.4%(w/v) yeast extract, 0.4%(w/v) glycerol, 17mM potassium dihydrogenphosphate, 72mM dipotassium hydrogenphosphate) containing 50μg/ml of ampicillin. The obtained culture medium was transferred to 100ml of TB medium containing 50μg/ml of ampicillin and cultured at 26℃, so that the OD660 was 0.2. When OD660 reached about 2.0, 5-aminolevulinic acid was added to the final concentration of 500μM, and the culturing was continued. Thirty (30) minutes thereafter, IPTG was added to a final concentration of 200μM, and there was further culturing for 5 hours.

The cells were recovered from each of the culture mediums, washed with 0.1M tris-HCl buffer (pH7.5) and suspended in 10ml of said buffer containing 1mM PMSF. The obtained cell suspensions were subjected 6 times to a sonicator (Sonifier (Branson

Sonic Power Company)) at 3 minutes each under the conditions of output 3, duty cycle 30%, in order to obtain cell lysate solutions. After centrifuging the cell lysate solutions (1,200xg, 5 minutes) the supernatants were recovered and centrifuged (150,000xg, 70 minutes) to recover supernatant fractions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSNSCA is referred to as "E. coli pKSNSCA extract ").

(3) **Detection of the ability to convert compound (II) to compound (III)**

Reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. The reaction solutions consisted of a 0.1M potassium phosphate buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 2mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 0.1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 18μl of the supernatant fraction recovered in Example 14(2). Further, there were prepared and maintained similarly reaction solutions having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B and component C. Three microliters (3μl) of 2N HCl and 90μl of ethyl acetate were added and stirred into each of the reaction solutions after the maintenance. The resulting reaction solutions were centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof was spotted to a silica gel TLC plate (TLC plate silica gel $60F_{254}$, 20cm x 20cm, 0.25mm thick, Merck Company). The TLC plate was developed with 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents were then allowed to evaporate. The TLC plate was exposed overnight to an imaging plate (Fuji

Film Company). Next, the imaging plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). The results are shown in Table 12.

Table 12

Reaction components					spot of compound (III)
component A	component B	component C	E. coli extract	compound (II) labeled with 14 C	
+	+	+	−	+	−
+	+	+	pKSNSCA	+	+

Example 15 Isolation of Soybean RuBPC Gene

After seeding soybean (cv. Jack), the soybean was cultivated at 27℃ for 30 days and the leaves were gathered. Two-tenths grams (0.2g) to 0.3g of the gathered leaves were frozen with liquid nitrogen and were milled with a mortar and pestle. Subsequently, the total RNA was extracted from the milled product according to the manual attached with RNA extraction solvent ISOGEN (Nippon Gene Company). Further, cDNA was synthesized with the use of Superscript First-strand Synthesis System for RT-PCR (Invitrogen Company), by conducting the procedures in accordance with the attached manual. Specifically, a 1st strand cDNA was synthesized by utilizing the Oligo(dT)$_{12-18}$ primer provided by the kit as a primer and the total soybean RNA as the template and by adding thereto the reverse transcriptase provided by the kit. Next, there is amplified by PCR a DNA encoding the chloroplast transit peptide of the small subunit of ribulose-1,5-bisphosphate carboxylase (hereinafter, the ribulose-1,5-bisphosphate carboxylase is referred to as "RuBPC") of soybean (cv. Jack) followed by the 12 amino acids of a mature protein (hereinafter, the chloroplast transit peptide of the small subunit of RuBPC

of soybean (cv. Jack) is sometimes referred to as "rSt"; and the DNA encoding the chloroplast transit peptide of the small subunit of RuBPC of soybean (cv. Jack) followed by the 12 amino acids of a mature protein is referred to as "the present rSt12 DNA"). The PCR utilized the obtained cDNA as a template and as primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 86 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 87. The PCR utilized LA Taq polymerase (Takara Shuzo Company). The PCR was conducted by maintaining once 94℃ for 3 minutes; conducting 30 cycles of a cycle that included maintaining 98℃ for 25 seconds and then 68℃ for 1 minute; and maintaining once 72℃ for 10 minutes. Plasmid pCRrSt12 (Fig. 5) was obtained by inserting the amplified DNA into the PCR-product cloning site of plasmid pCR2.1 (Invitrogen Company). Next, plasmid was introduced into the competent cells of E. coli JM109 strain and the ampicillin resistant strains were selected. Further, the nucleotide sequence of the plasmid contained in the selected ampicillin resistant strains was determined by utilizing the Dye Terminator Cycle Sequencing FS Ready Reaction kit (PE Applied Biosystems Company) and the DNA sequencer 373S (PE Applied Biosystems Company). As a result, the nucleotide sequence shown in SEQ ID NO: 88 was provided. It was confirmed that plasmid pCRrSt12 contained the present rSt12 DNA.

Example 16 Construction of a Chloroplast Expression Plasmid Containing the Present Invention DNA (A1) for Direct Introduction

(1) Isolation of the present invention DNA (A1)

A DNA comprising the nucleotide sequence shown in SEQ ID NO: 6 was amplified by PCR. The PCR was conducted by utilizing as the template the genomic DNA of Actinomyces Streptomyces phaeochromogenes IFO12898 and by utilizing as

primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 93 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 94. Further, a DNA comprising the nucleotide sequence shown in SEQ ID NO: 9 was amplified by PCR. The PCR was conducted by utilizing as primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 93 and the oligonucleotide sequence shown in SEQ ID NO: 95. Said PCR utilized the Expand High Fidelity PCR System (Boehringer Company). There was conducted after maintaining once 97℃ for 2 minutes; conducting 10 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 1 minute; then conducting 15 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 1 minute (wherein 20 seconds were added to the maintenance at 72℃ for each cycle); and then maintaining 72℃ for 7 minutes. Plasmids pCR657ET (Fig. 6) and pCR657FET (Fig. 7) were produced by inserting the amplified DNA into the PCR product cloning region of pCR2.1 (Invitrogen Company).

Furthermore, other than utilizing the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 96 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 94, plasmid pCR657Bs (Fig. 8) was obtained with procedures similar to the method described above. Even further, other than utilizing the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 96 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 97, plasmid pCR657FBs (Fig. 9) was obtained with procedures similar to the method described above. Next, the plasmids were introduced into E. Coli DH5 α competent cells (Takara Shuzo Company) and the ampicillin resistant cells were selected. Further, the nucleotide sequences of the plasmids contained in the ampicillin resistant strains were determined by utilizing BigDye Terminator Cycle Sequencing Ready Reaction kit v2.0 (PE Applied

Biosystems Company) and DNA sequencer 3100 (PE Applied Biosystems Company). As a result, it was confirmed that plasmids pCR657ET and pCR657Bs have the nucleotide sequence shown in SEQ ID NO: 6. It was confirmed that plasmids pCR657FET and pCR657FBs have the nucleotide sequence shown in SEQ ID NO: 9.

(2) Construction of a chloroplast expression plasmid having the present invention DNA (A1) for direct introduction - part (1)

A plasmid containing a chimeric DNA in which the present invention DNA (A1) was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit (hereinafter sometimes referred to as the "sequence encoding the chloroplast transit peptide") without a change of frames in the codons was constructed as a plasmid for introducing the present invention DNA (A1) into a plant with the particle gun method.

First, pCRrSt12 was digested with restriction enzyme HindIII and KpnI. The DNA comprising the present rSt12DNA was isolated. Further, a DNA of about 2640bp was obtained by removing about a 40bp DNA from plasmid vector pUC19 (Takara Shuzo Company) with a digestion with restriction enzymes HindIII and KpnI. Next, the 5' terminus of the DNA was dephosphorylated with calf intestine alkaline phosphatase (Takara Shuzo Company). The DNA containing the present rSt12DNA, obtained from pCRrSt12, was inserted thereto to obtain pUCrSt12 (Fig. 10). Next, DNA comprising the present invention DNA (A1) were isolated by digesting each of plasmids pCR657ET and pCR657FET with restriction enzymes EcoT22I and SacI. Each of the obtained DNA was inserted between the EcoT22I restriction site and the SacI restriction site of pUCrSt12 to obtain plasmids pUCrSt657 (Fig. 11) and pUCrSt657F (Fig. 12) containing a chimeric DNA in which the present invention DNA (A1) was connected immediately after the nucleotide

sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit without a change of frames in the codons.

pBICR16G6PT (described in Japanese unexamined patent 2000-166577) was digested with restriction enzyme EcoRI to isolate a DNA of about 3kb. (Hereinafter, the promoter contained in the DNA described in the above Japanese unexamined patent is referred to as the "CR16G6 promoter". Further, the terminator contained in the DNA described in the above Japanese unexamined patent is referred to as the "CR16 terminator".) After digesting the plasmid vector pUC19 (Takara Shuzo Company) with restriction enzyme EcoRI, the 5' terminus of said DNA was dephosphorylated with calf intestine alkaline phosphatase (Takara Shuzo Company). The 3kb DNA derived from pBICR16G6PT was inserted thereto to obtain plasmid pUCCR16G6-p/t (Fig. 13). pUCCR16G6-p/t was digested with restriction enzymes HindIII and ScaI to isolate a DNA comprising the CR16G6 promoter. Further, by digesting plasmid vector pUC19 (Takara Shuzo Company) with restriction enzymes HindIII and EcoRI, a DNA of 51bp was removed and the remaining DNA consisting of 2635bp was obtained. Next, the 5' terminus of said DNA was dephosphorylated with calf intestine alkaline phosphatase (Takara Shuzo Company). The above DNA comprising the CR16G6 promoter obtained from pUCCR16G6-p/t and a NotI-EcoRI linker (Fig. 14) obtained from annealing the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID No: 89 with the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID No: 90 were inserted thereto to obtain pUCCR12G6-p/t Δ (Fig. 15). pUCCR12G6-p/t Δ was digested with restriction enzymes NdeI and EcoRI to isolate a DNA having a partial nucleotide sequence of the CR16t terminator. Further, plasmid vector pUC19 (Takara Shuzo Company) was digested with restriction enzymes HindIII and EcoRI to obtain a DNA of 2635bp. The 5' terminus of said DNA was dephosphorylated with calf intestine alkaline

phosphatase (Takara Shuzo Company). The above DNA having a partial nucleotide sequence of the CR16t terminator obtained from pUCCR12G6-p/t Δ and a HindIII-NotI linker (Fig. 16) obtained by annealing the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 91 with the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 92 were inserted thereto to obtain pNdG6- Δ T (Fig. 17).

Next, by digesting each of plasmids pUCrSt657 and pUCr657F with restriction enzymes BamHI and SacI, there was isolated the DNA comprising a chimeric DNA in which the present invention DNA (A1) was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit without a change of frames in the codons. The DNA were inserted between the restriction enzyme site of BglII and the restriction enzyme site of SacI of plasmid pNdG6- Δ T to obtain each of plasmid pSUM-NdG6-rSt-657 (Fig. 18) and plasmid pSUM-NdG6-rSt-657F (Fig. 19).

(3) Construction of a chloroplast expression plasmid having the present invention DNA (A1) for direct introduction - part (2)

A plasmid containing a chimeric DNA in which the present invention DNA (A1) was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frames in the codons was constructed as a plasmid for introducing the present invention DNA (A1) into a plant with the particle gun method. First, after digesting plasmid vector pKF19 (Takara Shuzo Company) with restriction enzyme BspHI, the DNA termini were blunt ended by adding nucleotides to the double stranded gap, utilizing KOD DNA polymerase (Toyobo Corporation). Plasmid pKF19 Δ Bs was obtained by a self-cyclizing the resulting DNA with T4 DNA

ligase. The pCRrSt12 obtained in Example 1 was digested with restriction enzyme HindIII and KpnI. The DNA comprising the present rSt12DNA was isolated. Plasmid pKF19 Δ Bs was digested with restriction enzymes HindIII and KpnI to obtain a DNA of about 2160bp. The 5' termini of said DNA were dephosphorylated with calf intestine alkaline phosphatase (Takara Shuzo Company). The DNA comprising the present rSt12DNA obtained from pCRrSt12 was inserted thereto to obtain pKFrSt12 (Fig. 20). Next, the plasmids pCR657Bs and pCR657FBs obtained in Example 16(1) were each digested with restriction enzymes BspHI and SacI to isolate DNA comprising the present invention DNA (A1). Each of these DNA were inserted between the restriction site of BspHI and restriction site of SacI of plasmid pKFrSt12 to obtain plasmid pKFrSt12-657 (Fig. 21) and plasmid pKFrSt12-657F (Fig. 22), which contained a chimeric DNA in which the present invention DNA (A1) was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frames in the codons.

Next, each of plasmids pKFrSt12-657 and pKFrSt12-657F was digested with BamHI and SacI to obtain DNA comprising the present invention DNA (A1). Each of these DNA were inserted between the BglII restriction site and SacI restriction site of plasmid pNdG6- Δ T obtained in Example 16(2) to obtain plasmids pSUM-NdG6-rSt12-657 (Fig. 23) and pSUM-NdG6-rSt12-657F (Fig. 24) wherein the chimeric DNA, in which the present invention DNA (A1) was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frames in the codons, was connected downstream of promoter CR16G6.

Example 17 Introduction of the Present Invention DNA (A1) into Soybean

(1) Preparation of proliferative somatic embryos

After dipping pods of soybeans (cultivar: Fayette and Jack) in 1% sodium hypochlorite solution to sterilize, the immature seeds were taken out. The seed coat was exfoliated from the seed to remove the immature embryo having a diameter of 2 to 5 mm. The embryonic axis of the obtained immature embryo was excised with a scalpel to prepare the immature cotyledon. The immature cotyledon was divided into 2 cotyledon parts. Each cotyledon part was placed in the somatic embryo development medium, respectively. The somatic embryo development medium was a solidified medium where 0.2%(w/v) Gelrite was added to Murashige-Skoog medium (described in Murashige T.and Skoog F., Physiol. Plant (1962) 15, p473; hereinafter referred to as "MS medium") that was set to a pH of 7.0 and that had 180μM of 2,4-D and 30g/L of sucrose added thereto. About 1 month after the placement, the formed globular embryo was transplanted to the somatic embryo growth medium. The somatic embryo growth medium was a solidified medium where 0.2%(w/v) Gelrite was added to MS medium that was set to pH5.8 and that had 90μM of 2,4-D and 30g/L of sucrose added thereto. The globular embryo was thereafter transplanted to fresh somatic embryo growth medium 5 to 8 times at intervals of 2 to 3 weeks. Each of the culturing conditions utilizing the above somatic embryo development medium and somatic embryo growth medium was 23 hours of light with 1 hour of darkness and 23 to 25℃ for the whole day.

(2) Introduction of the gene to proliferative somatic embryos

After the globular embryo obtained in Example 17(1) is transplanted to fresh somatic embryo growth medium and cultured for 2 to 3 days, the globular embryo was utilized to introduce the gene. Plasmids pSUM-NdG6-rSt657, pSUM-NdG6-rSt657F,

pSUM-NdG6-rSt12657 and pSUM-NdG6-rSt12657F were coated onto gold particles of a diameter of 1.0μm to conduct the gene introduction employing the particle gun method. The amount of the plasmids was 1.66μg for 1mg of the gold particles. After introducing the gene, the embryo was cultured further for 2 to 3 days. Each of the culturing conditions was 23 hours of light with 1 hour of darkness and 23 to 25℃ for the whole day.

(3) Selection of an somatic embryo with hygromycin

The globular embryo after introducing the gene obtained in Example 17(2) was transplanted to an somatic embryo selection medium. The somatic embryo selection medium was a solidified medium where 0.2%(w/v) Gelrite and 15mg/L of hygromycin were added to MS medium that was set to pH5.8 and that had 90μM of 2,4-D and 30g/L of sucrose added thereto. The surviving globular embryo was thereafter transplanted to fresh somatic embryo selection medium 5 to 8 times at intervals of 2 to 3 weeks. In that time, the somatic embryo selection medium was a solidified medium where 0.2%(w/v) Gelrite and 30mg/L of hygromycin were added to MS medium that was set to pH5.8 and that had 90μM of 2,4-D and 30g/L of sucrose added thereto. Each of the culturing conditions utilizing the above somatic embryo selection medium was 23 hours of light with 1 hour of darkness and 23 to 25℃ for the whole day.

(4) Selection of somatic embryo with compound (II)

The globular embryo after introducing the gene obtained in Example 17(2) was transplanted to an somatic embryo selection medium. The somatic embryo selection medium was a solidified medium where 0.2%(w/v) Gelrite and 0.1mg/L of compound (II) were added to MS medium that was set to pH5.8 and that had 90μM of 2,4-D and

30g/L of sucrose added thereto. The surviving globular embryo was thereafter transplanted to fresh somatic embryo selection medium 5 to 8 times at intervals of 2 to 3 weeks. In that time, the somatic embryo selection medium was a solidified medium where 0.2%(w/v) Gelrite and 0.3 to 1mg/L of compound (II) were added to MS medium that was set to pH5.8 and that had 90μM of 2,4-D and 30g/L of sucrose added thereto. Each of the culturing conditions utilizing the above somatic embryo selection medium was 23 hours of light with 1 hour of darkness and 23 to 25℃ for the whole day.

(5) Plant regeneration from the somatic embryo

The globular embryos selected in Example 17(3) or 17(4) are transplanted to development medium and are cultured for 4 weeks in 23 hours of light with 1 hour of darkness and at 23 to 25℃ for the whole day. The development medium is a solidified medium where 0.8% (w/v) of agar (Wako Pure Chemical Industries, Ltd., use for plant tissue cultures) is added to MS medium that is set to pH5.8 and that has 60g/L of maltose added thereto. White to yellow colored cotyledon-type embryos are obtained 6 to 8 weeks thereafter. These cotyledon-type embryos are transplanted to germination medium and cultured for 2 weeks. The germination medium is a solidified medium where 0.2% (w/v) of Gelrite was added to MS medium that is set to pH5.8 and has 30g/L of sucrose added thereto. As a result, there can be obtained a soybean that has developed leaves and has roots.

(6) Acclimation and cultivation of the regenerated plant

The soybean obtained in Example 17(5) is transplanted to gardening soil and acclimated in an incubation chamber of 23 hours of light with 1 hour of darkness and 23 to 25℃ for the whole day. Two (2) weeks thereafter, the rooted plant is transferred to a

pot having a diameter of 9cm and cultivated at room temperature. The cultivation conditions at room temperature are natural light conditions at 23℃ to 25℃ for the whole day. Two to four (2 to 4) months thereafter, the soybean seeds are gathered.

(7) Evaluation of the resistance to herbicidal compound (II)

Leaves of the regenerated plant are gathered and are split equally into 2 pieces along the main vein. Compound (II) is spread onto the full surface of one of the leaf pieces. The other leaf piece is left untreated. These leaf pieces are placed on MS medium containing 0.8% agar and allowed to stand at room temperature for 7 days in light place. Then, each leaf piece is grounded with pestle and mortar in 5 ml of 80% aqueous acetone solution to extract chlorophyll. The extract liquid is diluted 10 fold with 80% aqueous acetone solution and the absorbance is measured at 750 nm, 663nm and 645nm to calculate total chlorophyll content according to the method described by Mackenney G., J. Biol. Chem. (1941) 140, p 315. The degree of resistance to compound (II) can be comparatively evaluated by showing in percentiles the total chlorophyll content of the treated leaf piece with the total chlorophyll content of the untreated leaf piece.

Further, soil is packed into a plastic pot having a diameter of 10cm and a depth of 10cm. Seeds of the above-described plant are seeded and cultivated in a greenhouse. An emulsion is prepared by mixing 5 parts of compound (II), 6 parts of sorpol3005X (Toho chemicals) and 89 parts of xylene. A certain amount thereof was diluted with water containing 0.1% (v/v) of a sticking agent at a proportion of 1000L for 1 hectare and is spread uniformly with a spray-gun onto the all sides of the foliage from above the plant cultivated in the above pot. After cultivating the plants for 16 days in a greenhouse, the damage to the plants is investigated, and the resistance to compound (II) is evaluated.

Example 18 Construction of a Chloroplast Expression Plasmid Having the Present Invention DNA (A1) for Agrobacterium Introduction

A plasmid for introducing the present invention DNA (A1) into a plant with the agrobacterium method was constructed. First, after binary plasmid vector pBI121 (Clontech Company) was digested with restriction enzyme NotI, the DNA termini were blunt ended by adding nucleotides to the double stranded gap, utilizing DNA polymerase I (Takara Shuzo Corporation). T4 DNA ligase was utilized for self-cyclization. After the obtained plasmid was digested with restriction enzyme EcoRI, the DNA termini were blunt ended by adding nucleotides to the double stranded gap, utilizing DNA polymerase I (Takara Shuzo Corporation). T4 DNA ligase was utilized for self-cyclization to obtain plasmid pBI121 Δ NotIEcoRI. After digesting the plasmid with HindIII, the 5' DNA terminus of the obtained DNA was dephosphorylated with calf intestine alkaline phosphatase (Takara Shuzo Company). A HindIII-NotI-EcoRI linker (Fig. 25) obtained by annealing the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 98 with the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 99 was inserted thereto. Binary plasmid vector pBI121S (Fig. 26) was obtained by self-cyclization. Said plasmid has a structure in which the HindIII-NotI-EcoRI linker was inserted in a direction in which the HindIII restriction site, the NotI restriction site, and the EcoRI restriction site line up in turn from a location close to the β-glucuronidase gene.

Next, each of plasmids pSUM-NdG6-rSt-657 and pSUM-NdG6-rSt-657F was digested with restriction enzymes HindIII and EcoRI, to obtain from each thereof a chimeric DNA in which the present invention DNA (A1) was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv.

Jack) RuBPC small subunit without a change of frames in the codons. These DNA were inserted between the HindIII restriction site and EcoRI restriction site of the above binary plasmid vector pBI121S to obtain plasmids pBI-NdG6-rSt-657 (Fig. 27) and pBI-NdG6-rSt-657F (Fig. 28). Further, each of the above plasmids pSUM-NdG6-rSt12-657 and pSUM-NdG6-rSt12-657F was digested with restriction enzymes HindIII and EcoRI, to obtain from each a chimeric DNA in which the present invention DNA (A1) was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frames in the codons. These DNA were inserted between the HindIII restriction site and EcoRI restriction site of the above binary plasmid vector pBI121S to obtain plasmids pBI-NdG6-rSt12-657 (Fig. 29) and pBI-NdG6-rSt12-657F (Fig. 30).

Example 19 Introduction of the Present Invention DNA (A1) to Tobacco

The present invention DNA (A1) was introduced into tobacco with the agrobacterium method, utilizing plasmid pBI-NdG6-rSt-657, plasmid pBI-NdG6-rSt-657F, plasmid pBI-NdG6-rSt12-657 and plasmid pBI-NdG6-rSt12-657F, obtained in Example 18.

First, the plasmids pBI-NdG6-rSt-657, pBI-NdG6-rSt-657F, pBI-NdG6rSt12-657 and pBI-NdG6-rSt12-657F were introduced into Agrobacterium tumefaciens LBA4404 (Clontech Company), respectively. Transformed agrobacterium strains bearing pBI-NdG6-rSt-657, pBI-NdG6-rSt-657F, pBI-NdG6-rSt12-657 or pBI-NdG6-rSt12-657F were isolated by culturing the resultant transformants in LB agar medium (0.5% yeast extract, 1.0% Bacto tryptone, 0.5% NaCl) containing 300 mg/L streptomycin, 100 mg/L rifampicin and 25 mg/L kanamycin and by selecting the resistant colonies.

Then, according to the method described in Manual for Gene Manipulation of Plant (by Hirofumi UCHIMIYA, Kodan-sha Scientific, 1992), the gene was introduced into tobacco. Agrobacterium strains bearing the above plasmids were each cultured at 28°C overnight in LB medium containing 300 mg/L streptomycin, 100 mg/L rifampicin and 25 mg/L kanamycin, and then leaf pieces of tobacco (Nicotiana tabacum strain SR1) cultured sterilely were dipped in the liquid culture medium. The leaf pieces were planted and cultured at room temperature for 2 days in the light in MS agar medium (MS inorganic salts, MS vitamins, 3% sucrose and 0.8% agar; described in Murashige T. and Skoog F., Physiol. Plant. (1962) 15, p 473) containing 0.1 mg/L of naphthalene acetic acid and 1.0 mg/L of benzyl aminopurine. Then, the leaf pieces were washed with sterilized water and cultured for 7 days on MS agar medium containing 0.1 mg/L of naphthalene acetic acid, 1.0 mg/L of benzyl aminopurine and 500mg/L of cefotaxime. Next, the leaf pieces were transplanted and cultured in MS agar medium containing 0.1mg/L of naphthalene acetic acid, 1.0mg/L of benzyl aminopurine, 500mg/L of cefotaxime and 100mg/L of kanamycin. The culture was conducted continuously for 4 months while transplanting the leaf pieces to fresh medium of the same composition at intervals of 4 weeks. At that time, the unfixed buds developing from the leave pieces were transplanted and rooted in MS agar medium containing 300mg/L of cefotaxime and 50mg/L of kanamycin to obtain regenerated bodies. The regenerated bodies were transplanted to and cultured in MS agar medium containing 50mg/L of kanamycin to obtain, respectively, a transgenic tobacco to which the T-DNA region of pBI-NdG6-rSt-657, pBI-NdG6-rSt-657F, pBI-NdG6-rSt12-657 or pBI-NdG6-rSt12-657F has been introduced.

Further, the plasmid pBI121S obtained in Example 18 was introduced into tobacco with the agrobacterium method. A transformed agrobacterium strain bearing pBI121S was isolated similarly to the above, other than utilizing plasmid pBI121S

instead of pBI-NdG6-rSt-657, pBI-NdG6-rSt-657F, pBI-NdG6-rSt12-657 and pBI-NdG6-rSt12-657F. Next, a transgenic tobacco to which the T-DNA region of plasmid pBI121S has been introduced was obtained similarly to the above, utilizing said transformed agrobacterium.

Three (3) leaves were taken from the transgenic tobacco. Each leaf was divided into 4 pieces in which each piece was 5 to 7mm wide. Each of the leaf pieces were planted onto MS agar medium containing 0.1mg/L of compound (II) and cultured in the light at room temperature. On the 7th day of culturing, the herbicidal damage of each of the leaf pieces was observed. The leaf pieces derived from the tobacco to which the control DNA (T-DNA region of plasmid pBI121S) was introduced turned white and withered. In contrast, the leaf pieces derived from the tobacco to which the present invention DNA (A1) (the T-DNA region of plasmid p pBI-NdG6-rSt-657, plasmid pBI-NdG6-rSt12-657,

pBI-NdG6-rSt-657F or pBI-NdG6-rSt12-657F) was introduced grew continuously.

Example 20 Introduction of the Present Invention DNA into a Plant

Plasmids were constructed for introducing the present invention DNA (A2) with the particle gun method and the agrobacterium method. First, the present invention DNA (A2) having the nucleotide sequence shown in SEQ ID NO: 7 was amplified by PCR. The PCR was conducted by utilizing as the template the genomic DNA of Actinomyces Saccharopolyspora taberi JCM9383t and by utilizing as primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 100 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 101. Said PCR utilized the Expand High Fidelity PCR System (Boehringer Company). There were conducted after maintaining once 97℃ for 2 minutes; repeating 10 cycles of a cycle that included

maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 60 seconds; then conducting 15 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 1 minute (wherein 20 seconds were added to the maintenance at 72℃ for each cycle); and then maintaining 72℃ for 7 minutes. Plasmids pCR923Sp (Fig. 31) was produced by inserting the amplified DNA into the PCR product cloning region of pCR2.1-TOPO (Invitrogen Company). Next, the plasmid was introduced into E. Coli JM109 competent cells (Takara Shuzo Company) and the ampicillin resistant cells were selected. Further, the nucleotide sequences of the plasmids contained in the ampicillin resistant strains were determined by utilizing BigDye Terminator Cycle Sequencing Ready Reaction kit v2.0 (PE Applied Biosystems Company) and DNA sequencer 373S (PE Applied Biosytems Company). As a result, it was confirmed that plasmid pCR923Sp has the nucleotide sequence shown in SEQ ID NO: 7.

Plasmid pKFrSt12, designed in Example 16(3), was digested with restriction enzymes BamHI and SacI to isolate a DNA comprising the present rSt12DNA. Said DNA was inserted between the BglII restriction site and SacI restriction site of pNdG6-ΔT obtained in Example 16(2) to obtain plasmid pNdG6-rSt12 (Fig. 32). Plasmid pCR923Sp was digested with restriction enzymes SphI and KpnI to obtain the DNA comprising the present invention DNA (A2). Plasmid pNdG6-rSt12 was digested with restriction enzymes SphI and KpnI to remove the DNA encoding the 12 amino acids of the mature protein of soybean (cv. Jack) RuBPC small subunit. In its place, the above DNA containing the present invention DNA (A2) obtained from plasmid pCR923Sp was inserted to obtain pSUM-NdG6-rSt-923 (Fig. 33) wherein the CR16G6 promoter has connected downstream therefrom the chimeric DNA in which said DNA was connected immediately after the sequence encoding the chloroplast transit peptide of soybean (cv.

Jack) RuBPC small subunit, without a change of frame in the codons.

Next, plasmid pCR923Sp was digested with restriction enzyme SphI. After blunting the ends of the obtained DNA with KOD DNA polymerase, said DNA is further digested with restriction enzyme KpnI to isolate a DNA containing the present invention DNA (A2). Plasmid pKFrSt12 produced in Example 16(3) was digested with restriction enzyme BspHI. After blunting the ends of the obtained DNA with KOD DNA polymerase, said DNA is further digested with restriction enzyme KpnI to remove DNA of about 20bp. In its place, the above DNA containing the present invention DNA (A2) obtained from plasmid pCR923Sp was inserted to obtain plasmid pKFrSt12-923 (Fig. 34) comprising the chimeric DNA in which the present invention DNA (A2) was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frames in the codons. pKFrSt12-923 was digested with restriction enzymes SphI and KpnI to obtain the chimeric DNA in which the present invention DNA (A2) and the DNA encoding the first 12 amino acids of the mature protein of soybean (cv. Jack) RuBPC small subunit are connected. Plasmid pNdG6-rSt12 was digested with restriction enzymes SphI and KpnI to remove the DNA encoding the 12 amino acids of the mature protein of soybean (cv. Jack) RuBPC small subunit. In its place, the above chimeric DNA obtained from plasmid pKFrSt12-923 was inserted to obtain plasmid pSUM-NdG6-rSt12-923 (Fig. 35) in which the CR16G6 promoter has connected downstream therefrom the chimeric DNA in which said DNA containing the present invention DNA (A2) was connected immediately after the sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frame in the codons.

The present invention DNA (A2) was introduced into soybean with the particle

gun method with the identical procedures of the method described in Example 17, utilizing the obtained plasmids pSUM-NdG6-rSt-923 and pSUM-NdG6-rSt12-923.

The above plasmid pSUM-NdG6-rSt-923 was digested with restriction enzymes HindIII and EcoRI to isolate the DNA comprising the chimeric DNA in which said DNA containing the present invention DNA (A2) was connected immediately after the sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit, without a change of frame in the codons. As in producing pBI-NdG6-rSt657 in Example 18, the above DNA containing the chimeric DNA obtained from plasmid pSUM-NdG6-rSt-923 was inserted between the HindIII restriction site and the EcoRI restriction site of binary vector pBI121S to obtain pBI-NdG6-rSt-923 (Fig. 36). Further, the above plasmid pSUM-NdG6-rSt12-923 was digested with HindIII and EcoRI, to isolate the DNA containing chimeric DNA in which said DNA containing the present invention DNA (A2) was connected immediately after the sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frame in the codons. The chimeric DNA obtained from pSUM-NdG6-rSt12-923 was inserted between the HindIII restriction site and EcoRI restriction sites of binary vector pBI121S to obtain pBI-NdG6-rSt12-923 (Fig. 37).

Each of the plasmids pBI-NdG6-rSt-923 and pBI-NdG6-rSt12-923 was introduced into Agrobacterium tumefaciens LBA4404. The resultant transformants were cultured in LB medium containing 300μg/ml of streptomycin, 100μg/ml of rifampicin and 25μg/ml of kanamycin. The transformants were selected to isolate agrobacterium strains bearing pBI-NdG6-rSt-923 or pBI-NdG6-rSt12-923.

Leaf pieces of sterily cultured tobacco were infected with each of the agrobacterium strain bearing pBI-NdG6-rSt-923 and the agrobacterium strain bearing

pBI-NdG6-rSt12-923. Tobaccos in which the present invention DNA (A2) has been introduced were obtained under the procedures similar to the methods described in Example 19.

Three (3) leaves were taken from the obtained transgenic tobacco. Each leaf was divided into 4 pieces in which each piece was 5 to 7mm wide. Each of the leaf pieces were planted onto MS agar medium containing 0.1mg/L of compound (II) and cultured in the light at room temperature. On the 7th day of culturing, the herbicidal damage of each of the leaf pieces was observed. The leaf pieces derived from the tobacco to which the control DNA (T-DNA region of plasmid pBI121S) was introduced turned white and withered. In contrast, the leaf pieces derived from the tobacco to which the present invention DNA (A2) (the T-DNA region of plasmid pBI-NdG6-rSt923 or plasmid pBI-NdG6-rSt12-923) was introduced grew continuously.

Example 21 Introduction of the Present Invention DNA (A3) into Tobacco

Plasmids were constructed for introducing the present invention DNA (A3) into a plant with the particle gun method and with the agrobacterium method.

First, the present invention DNA (A3) having the nucleotide sequence shown in SEQ ID NO: 8 was amplified by PCR. The PCR was conducted by utilizing as the template the genomic DNA of Actinomyces Streptomyces testaceus ATCC21469 and by utilizing as primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 102 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 103. Said PCR utilized the Expand High Fidelity PCR System (Boehringer Company). There were conducted after maintaining once 97℃ for 2 minutes; repeating 10 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 1 minute; then conducting 15 cycles of a cycle that

included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 1 minute (wherein 20 seconds were added to the maintenance at 72℃ for each cycle); and then maintaining once 72℃ for 7 minutes. Plasmid pCR671ET (Fig. 38) was produced by inserting the amplified DNA into the PCR product cloning region of pCR2.1 (Invitrogen Company). Further, plasmid pCR671Bs (Fig. 39) was obtained with the procedures similar to the method described above, other than utilizing as the PCR primers, the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 104 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 103. Next, the plasmids were introduced into E. Coli JM109 competent cells (Takara Shuzo Company) and the ampicillin resistant cells were selected. Further, the nucleotide sequences of the plasmids contained in the ampicillin resistant strains were determined by utilizing BigDye Terminator Cycle Sequencing Ready Reaction kit v2.0 (PE Applied Biosystems Company) and DNA sequencer 3100 (PE Applied Biosytems Company). As a result, it was confirmed that plasmids pCR671ET and pCR671Bs have the nucleotide sequence shown in SEQ ID NO: 8.

Plasmid pCR671ET was digested with restriction enzymes EcoT22I and KpnI to isolate DNA comprising the present invention DNA (A3). Said DNA was inserted between the EcoT22I restriction site and the KpnI restriction site to obtain plasmid pUCrSt671 (Fig. 40) comprising the chimeric DNA in which the present invention DNA (A3) was connected immediately after the sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit, without a change of frame in the codons. Plasmid pUCrSt671 was digested with restriction enzymes NheI and KpnI to isolate DNA comprising the present invention DNA (A3). Plasmid pNdG6-rSt12, obtained in Example 16(2), was digested with restriction enzymes NheI and KpnI to remove DNA of about 80bp. In its place, the above DNA containing the present

invention DNA (A3) obtained from plasmid pUCrSt671 was inserted to obtain pSUM-NdG6-rSt-671 (Fig. 41) wherein the CR16G6 promoter has connected downstream therefrom the chimeric DNA in which the present invention DNA (A3) was connected immediately after the sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit, without a change of frame in the codons.

Plasmid pCR671Bs was digested with restriction enzymes BspHI and KpnI to isolate a DNA comprising the present invention DNA (A3). Said DNA was inserted between the BspHI restriction site and KpnI restriction site of pKFrSt12 obtained in Example 16(3) to obtain plasmid pKFrSt12-671 (Fig. 42) containing the chimeric DNA in which the present invention DNA (A3) was connected immediately after the sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frame in the codons. Plasmid pNdG6-rSt12 obtained in Example 20 was digested with restriction enzymes NheI and KpnI to remove DNA of about 80bp. In its place, the above DNA containing the present invention DNA (A3) obtained from plasmid pKFrSt12-671 was inserted to obtain pSUM-NdG6-rSt12-671 (Fig. 43) wherein the CR16G6 promoter has connected downstream therefrom the chimeric DNA in which the present invention DNA (A3) was connected immediately after the sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frame in the codons.

The present invention DNA (A3) was introduced into soybean with the particle gun method with procedures similar to the method described in Example 17, utilizing the obtained plasmids pSUM-NdG6-rSt-671 and pSUM-NdG6-rSt12-671.

The above plasmid pSUM-NdG6-rSt-671 was digested with restriction enzymes HindIII and EcoRI to isolate the chimeric DNA in which the present invention DNA (A3)

was connected immediately after the sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit, without a change of frame in the codons. The above DNA containing the chimeric DNA obtained from plasmid pSUM-NdG6-rSt-671 was inserted between the HindIII restriction site and the EcoRI restriction site of binary vector plasmid pBI121S obtained in Example 18, to obtain pBI-NdG6-rSt-671 (Fig. 44). Further, the above plasmid pSUM-NdG6-rSt12-671 was digested with restriction enzymes HindIII and EcoRI, to isolate the DNA containing chimeric DNA in which said DNA containing the present invention DNA (A3) was connected immediately after the sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frame in the codons. The chimeric DNA obtained from pSUM-NdG6-rSt12-671 was inserted between the HindIII restriction site and EcoRI restriction sites of binary plasmid vector pBI121S to obtain pBI-NdG6-rSt12-671 (Fig. 45).

Each of the plasmids pBI-NdG6-rSt-671 and pBI-NdG6-rSt12-671 were introduced into Agrobacterium tumefaciens LBA4404. The resultant transformants were cultured in LB medium containing 300μg/ml of streptomycin, 100μg/ml of rifampicin and 25μg/ml of kanamycin. The transformants were selected to isolate agrobacterium strains bearing pBI-NdG6-rSt-671 or pBI-NdG6-rSt12-671.

Leaf pieces of sterily cultured tobacco were infected with each of the agrobacterium strain bearing pBI-NdG6-rSt-671 and the agrobacterium strain bearing pBI-NdG6-rSt12-671. Tobaccos in which the present invention DNA (A3) has been introduced were obtained under the procedures similar to the methods described in Example 19.

Three (3) leaves are taken from the transgenic tobaccos. Each leaf is divided into 4 pieces in which each piece was 5 to 7mm wide. Each of the leaf pieces are planted onto

MS agar medium containing 0.1mg/L of compound (II) and cultured in the light at room temperature. On the 7th day of culturing, the herbicidal damage of each of the leaf pieces is observed.

Example 22 Expression of the Present Invention Protein (B1) in E. Coli

(1) Production of a transformed E. coli of the present invention DNA (B1)

PCR was conducted by utilizing as a template the chromosomal DNA prepared from Streptomyces phaeochromogenes IFO12898 in Example 3(1). The PCR reaction solution amounted to 50μl by adding 300ng of the above chromosomal DNA, 4μl of dNTP mix (a mixture of 2.5mM of each of the 4 types of dNTP), 5μl of 10x ExTaq buffer, 0.5μl of ExTaq polymerase (Takara Shuzo Company), distilled water and 200nM of each of the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 105 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 53. The reaction conditions of the PCR were after maintaining 97℃ for 2 minutes; repeating 25 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 90seconds; and then maintaining 72℃ for 4 minutes. The reaction solution after the maintenance and the vector pCR2.1-TOPO (Invitrogen Company) were ligated according to the instructions attached to said vector and were introduced into E. Coli TOP10F'. The plasmid DNA were prepared from the obtained E. coli transformants, utilizing QIAprep Spin Miniprep Kit (Qiagen Company). Sequencing reactions were conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 67 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 68. The sequencing reactions utilized the obtained plasmid DNA as the template.

The reaction products were analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). Based on the results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 15 was designated as pCR657FD.

Next, pCR657FD was digested with restriction enzymes NdeI and HindIII. The digestion products were subjected to agarose gel electrophoresis. The gel area containing a DNA of about 200bp was cut from the gel. The DNA was purified from the recovered gels by utilizing QIA quick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated with ligation kit Ver.1 (Takara Shuzo Company) according to the instructions attached to said kit and introduced into E. Coli JM109. The plasmid DNA were prepared from the obtained E. coli transformants. The structures thereof were analyzed. The plasmid containing the nucleotide sequence shown in SEQ ID NO: 15, in which the DNA of about 200bp encoding the present invention protein (B1) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN657FD. The plasmid pKSN657FD was introduced into E. coli JM109. The obtained E. coli transformant was designated JM109/pKSN657FD. Further, plasmid pKSN2 was introduced into E. coli JM109. The obtained E. coli transformant was designated as JM109/pKSN2.

(2) Expression of the present invention protein (B1) in E. coli and recovery of said protein

E. coli JM109/pKSN657FD and E. Coli JM109/pKSN2 were each cultured overnight at 37℃ in 10ml of TB medium (1.2%(w/v) tryptone, 2.4%(w/v) yeast extract, 0.4%(w/v) glycerol, 17mM potassium dihydrogenphosphate, 72mM dipotassium hydrogenphosphate) containing 50μg/ml of ampicillin. A milliliter (1ml) of the obtained

culture medium was transferred to 100ml of TB medium containing 50μg/ml of ampicillin and cultured at 26℃. Thirty (30) minutes after the OD660 reached about 0.5, IPTG was added to a final concentration of 1mM, and there was further culturing for 20 hours.

The cells were recovered from each of the culture mediums, washed with 0.1M tris-HCl buffer (pH7.5) and suspended in 10ml of said buffer containing 1mM PMSF. The obtained cell suspensions were subjected 6 times to a sonicator (Sonifier (Branson Sonic Power Company)) at 3 minutes each under the conditions of output 3, duty cycle 30%, in order to obtain cell lysate solutions. After centrifuging the cell lysate solutions (1,200xg, 5 minutes) the supernatants were recovered and centrifuged (150,000xg, 70 minutes) to recover supernatant fractions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN657FD is referred to as "E. coli pKSN657FD extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract"). A microliter (1μl) of the above supernatant fractions was analyzed on a 15% to 25% SDS-PAGE and stained with CBB. As a result, notably more intense bands were identified in the E. coli pKSN657FD extract than the E. coli pKSN2 extract, at the electrophoresis locations corresponding to the molecular weight of 7kDa. It was shown that E. coli JM109/pKSN657FD expressed the present invention protein (B1).

(3) **Use of the present invention protein (B1) for a reaction system of converting compound (II) to compound (III)**

Reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. The reaction solutions consisted of a 0.1M potassium phosphate buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 9μl of the E. coli pKSN657FD

extract recovered in Example 22(2), 0.1U/ml of ferredoxin reductase (hereinafter referred to as "component C") (Sigma Company) and 15μl of the E. coli pKSN657F extract recovered in Example 4(2) (hereinafter referred to as "component D"). Further, there were prepared reaction solutions in which 2mg/ml of ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company) was added in the place of the E. coli pKSN657FD extract and a reaction solution in which nothing was added in the place of the E. coli pKSN657FD extract. Such reaction solutions were maintained similarly. Three microliters (3μl) of 2N HCl and 90 μl of ethyl acetate were added and mixed into each of the reaction solutions after the maintenance. The resulting reaction solutions were centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof was spotted to a silica gel TLC plate (TLC plate silica gel $60F_{254}$, 20cm x 20cm, 0.25mm thick, Merck Company). The TLC plate was developed with a 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents were then allowed to evaporate. The TLC plate was exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). The results are shown in Table 13.

Table 13

Reaction components						spot of
component A	E. coli extract	component B	component C	component D	compound (II) labeled with ^{14}C	compound (III)
+	pKSN657FD	−	+	+	+	+
+	−	−	+	+	+	−
+	−	+	+	+	+	+

Example 23 Expression of the Present Invention Protein (B2) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (B2)

PCR is conducted by utilizing as a template the chromosomal DNA prepared from Saccharopolyspora taberi JCM9383t in Example 6(1). The PCR reaction solution amounts to 50μl by adding 300ng of the above chromosomal DNA, 4μl of dNTP mix (a mixture of 2.5mM of each of the 4 types of dNTP), 5μl of 10x ExTaq buffer, 0.5μl of ExTaq polymerase (Takara Shuzo Company), distilled water and 200nM of each of the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 106 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 63. The reaction conditions of the PCR are after maintaining 97℃ for 2 minutes; repeating 25 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 90seconds; and then maintaining 72℃ for 4 minutes. The reaction solution after the maintenance and the vector pCR2.1-TOPO (Invitrogen Company) are ligated according to the instructions attached to said vector and introduced into E. Coli TOP10F'. The plasmid DNA are prepared from the obtained E. coli transformants, utilizing QIAprep Spin Miniprep Kit (Qiagen Company). Sequencing reactions are conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 67 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 68. The sequencing reactions utilize the obtained plasmid DNA as the template. The reaction products are analyzed with a DNA sequencer 373A (Applied Biosystems Japan Company). Based on the results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 16 is designated as pCR923FD.

Next, plasmid pCR923FD is digested with restriction enzymes NdeI and HindIII. The digestion products are subjected to agarose gel electrophoresis. The gel area containing a DNA of about 200bp is cut from the gel. The DNA is purified from the recovered gels by utilizing QIA quick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII are ligated with ligation kit Ver.1 (Takara Shuzo Company) according to the instructions attached to said kit and introduced into E. Coli JM109. The plasmid DNA are prepared from the obtained E. coli transformants. The structures thereof are analyzed. The plasmid containing the nucleotide sequence shown in SEQ ID NO: 16, in which the DNA of about 200bp encoding the present invention protein (B2) is inserted between the NdeI site and the HindIII site of pKSN2 is designated as pKSN923FD. The plasmid pKSN923FD is introduced into E. coli JM109. The obtained E. coli transformant is designated as JM109/pKSN923FD. Further, plasmid pKSN2 is introduced into E. coli JM109. The obtained E. coli transformant is designated as JM109/pKSN2.

(2) Expression of the present invention protein (B2) in E. coli and recovery of said protein

E. coli JM109/pKSN923FD and E. Coli JM109/pKSN2 are each cultured overnight at 37℃ in 10ml of TB medium (1.2%(w/v) tryptone, 2.4%(w/v) yeast extract, 0.4%(w/v) glycerol, 17mM potassium dihydrogenphosphate, 72mM of dipotassium hydrogenphosphate) containing 50μg/ml of ampicillin. A milliliter (1ml) of the obtained culture medium is transferred to 100ml of TB medium containing 50μg/ml of ampicillin and cultured at 26℃. Thirty (30) minutes after the OD660 reached about 0.5, IPTG is added to a final concentration of 1mM, and there is further culturing for 20 hours.

The cells are recovered from each of the culture mediums, washed with 0.1M tris-

HCl buffer (pH7.5) and suspended in 10ml of said buffer containing 1mM PMSF. The obtained cell suspensions are subjected 6 times to a sonicator (Sonifier (Branson Sonic Power Company)) at 3 minutes each under the conditions of output 3, duty cycle 30%, in order to obtain cell lysate solutions. After centrifuging the cell lysate solutions (1,200xg, 5 minutes) the supernatants are recovered and centrifuged (150,000xg, 70 minutes) to recover supernatant fractions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN923FD is referred to as "E. coli pKSN923FD extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract"). A microliter (1μl) of the above supernatant fractions is analyzed on a 15% to 25% SDS-PAGE and stained with CBB. By detecting notably more intense bands in the E. coli pKSN923FD extract than the E. coli pKSN2 extract, at the electrophoresis locations corresponding to the molecular weight of 7kDa, it is possible to confirm to E. coli expression of the present invention protein (B2).

(3) Use of the present invention protein (B2) for a reaction system of converting compound (II) to compound (III)

Reaction solutions of 30μl are prepared and maintained for 10 minutes at 30℃. The reaction solutions consist of a 0.1M potassium phosphate buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 9μl of the E. coli pKSN923FD extract recovered in Example 23(3), 0.1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 15μl of the E. coli pKSN657F extract recovered in Example 4(2) (hereinafter referred to as "component D"). Further, there are prepared reaction solutions in which 2mg/ml of ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company) is added in the place of the E. coli

pKSN923FD extract and a reaction solution in which nothing is added in the place of the E. coli pKSN923FD extract. Such reaction solutions are maintained similarly. Three microliters (3μl) of 2N HCl and 90 μl of ethyl acetate are added and mixed into each of the reaction solutions after the maintenance. The resulting reaction solutions are centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue is dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof is spotted to a silica gel TLC plate (TLC plate silica gel $60F_{254}$, 20cm x 20cm, 0.25mm thick, Merck Company). The TLC plate is developed with a 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents are then allowed to evaporate. The TLC plate is exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging plate is analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C are examined (Rf value 0.24 and 0.29). By confirming that compound (III) is produced in the reaction including component A, E. coli pKSN923FD extract, component C and component D, it can be confirmed that the present invention protein (B2) can be used instead of the ferredoxin derived from spinach in a reaction system of converting compound (II) to compound (III).

Example 24 Expression of the Present Invention Protein (B3) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (B3)

PCR is conducted similarly to the methods described in Example 23(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces testaceus ATCC 21469 in Example 11(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 107 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 72. Plasmid pCR671FD having the

nucleotide sequence shown in SEQ ID NO: 17 is obtained similarly to the method described in Example 23(1) utilizing the obtained reaction solution.

Next, utilizing said plasmid, plasmid pKSN671FD in which the present invention DNA (B3) is inserted between the NdeI site and HindIII site of pKSN2 is obtained similarly to the method described in Example 23(1). By introducing the plasmid into E. coli JM109, E. coli JM109/pKSN671FD having the present invention DNA (B3) can be obtained.

(2) Expression of the present invention protein (B3) in E. coli and recovery of said protein

Utilizing E. coli JM109/pKSN671FD, supernatant fractions (hereinafter referred to as "E. coli pKSN671FD extract") are recovered similarly to the method described in Example 23(2). A microliter (1μl) of the above supernatant fractions is analyzed on a 15% to 25% SDS-PAGE and stained with CBB. As a result, by detecting notably more intense bands in the E. coli pKSN671FD extract than the E. coli pKSN2 extract, at the electrophoresis location corresponding to the molecular weight of 7kDa, it is possible to confirm the expression of the present invention protein (B3) in E. coli.

(3) Use of the present invention protein (B3) for a reaction system of converting compound (II) to compound (III)

Other than utilizing E. coli pKSN671FD extract recovered in Example 24(2), the spot corresponding to compound (III) labeled with ^{14}C (Rf values 0.24 and 0.29) is confirmed similarly to the method described in Example 23(3). By confirming that compound (III) is produced in the reaction including component A, E. coli pKSN671FD extract, component C and component D, it can be confirmed that the present invention

protein (B3) can be used instead of the ferredoxin derived from spinach in a reaction system of converting compound (II) to compound (III).

Example 25 Preparation of the present invention protein (A4)

(1) Preparation of the crude cell extract

A frozen stock of Streptomyces achromogenes IFO12735 was added to 10ml of A medium (0.1%(w/v) of glucose, 0.5%(w/v) tryptone, 0.5%(w/v) yeast extract, 0.1%(w/v) of dipotassium hydrogenphosphate, pH7.0) in a large test tube and incubated with shaking at 30℃ for 1 day to obtain a pre-culture. Eight milliliters (8ml) of the pre-culture was added to 200ml of A medium and was incubated with rotary shaking in a 500ml baffled flask at 30℃ for 2 days. Cell pellets were recovered by centrifuging (3,000xg, 10 min.) the resulting culture. These cell pellets were suspended in 100ml of B medium (1%(w/v) glucose, 0.1% beef extract, 0.2%(w/v) tryptose) containing compound (II) at 100ppm and were incubated with reciprocal shaking in a 500ml Sakaguchi flask for 20 hours at 30℃. Cell pellets were recovered by centrifuging (3,000xg, 10 min.) 2L of the resulting culture. The resulting cell pellets were washed twice with 1L of 0.1M potassium phosphate buffer (pH7.0) to provide 136g of the cell pellets.

These cell pellets were suspended in 0.1M potassium phosphate buffer (pH7.0) at 1ml to 2ml for 1g of the cell pellets. A millimolar of (1mM) PMSF, 5mM of benzamidine HCl, 1mM of EDTA, 3μg/ml of leupeptin, 3μg/ml of pepstatin and 1mM of dithiotritol were added to the cell suspension. A cell lysate solution was obtained by disrupting twice repetitively the suspension with a French press (1000kg/cm^2) (Ohtake Seisakusho). After centrifuging the cell lysate solution (40,000xg, 30 minutes), the supernatant was recovered and centrifuged for 1 hour at 150,000xg to recover the supernatant (hereinafter referred to as the "crude cell extract")

(2) **Determination of the ability of converting compound (II) to compound (III)**

There was prepared 30µl of a reaction solution consisting of 0.1M potassium phosphate buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2.4mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 0.5mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 15µl of the crude cell extract recovered in Example 25(1). The reaction solution was maintained at 30℃ for a hour. Further, there was prepared and maintained similarly a reaction solution having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B and component C. Three microliters (3µl) of 2N HCl and 90 µl of ethyl acetate were added and mixed into each of the reaction solutions after the maintenance. The resulting reaction solutions were centrifuged at 8,000xg to recover 75µl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 6.0µl of ethyl acetate. Five microliters (5.0µl) thereof was spotted to a silica gel TLC plate (TLC plate silica gel $60F_{254}$, 20cm x 20cm, 0.25mm thick, Merck Company). The TLC plate was developed with a 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents were then allowed to evaporate. The TLC plate was exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). The results are shown in Table 14.

Table 14

Reaction components					spot of compound (III)
component A	component B	component C	crude cell extract	compound (II) labeled with ^{14}C	
+	+	+	−	+	−
+	+	+	+	+	+
−	+	+	+	+	−
+	−	−	+	+	−

(3) **Fractionation of the crude cell extract**

Ammonium sulfate was added to the crude cell extract obtained in Example 25(1) to amount to 45% saturation. After stirring in ice-cooled conditions, the supernatant was recovered by centrifuging for 30 minutes at 12,000xg. After adding ammonium sulfate to the obtained supernatant to amount to 55% saturation and stirring in ice-cooled conditions, a pellet was recovered by centrifuging for 10 minutes at 12,000xg. The pellet was dissolved with 12.5ml of 20mM bistrispropane buffer (pH7.0). This solution was subjected to a PD10 column (Amersham Pharmacia Company) and eluted with 20mM of bistrispropane buffer (pH7.0) to recover 17.5ml of fractions containing proteins (hereinafter referred to as the "45-55% ammonium sulfate fraction").

(4) **Isolation of the present invention protein (A4)**

The 45-55% ammonium sulfate fraction prepared in Example 25(3) was injected into a HiLoad26/10 Q Sepharose HP column (Amersham Pharmacia Company). Next, after flowing 100ml of 20mM bistrispropane buffer (pH7.0) into the column, 20mM bistrispropane buffer was flown with a linear gradient of NaCl (gradient of NaCl was 0.004M/minute, range of NaCl concentration was from 0M to 1M, flow rate was 4ml/minute) to fraction recover 30ml of fractions eluting at the NaCl concentration of from 0.12M to 0.165M. Further, the recovered fractions were subjected to a PD10

column (Amersham Pharmacia Biotech Company) and eluted with 20mM bistrispropane buffer (pH7.0) to recover the fractions containing protein.

The recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) with the elution with Buffer A (2mM potassium phosphate buffer containing 1.5mM of NaCl, pH 7.0), in order to recover the fractions containing protein. Next, the fractions were injected into a Bio-Scale Ceramic Hydroxyapatite Type I column CHT10-I (BioRad Company). Twenty milliliters (20ml) of Buffer A was flown into the column. Subsequently, Buffer A was flown with a linear gradient of Buffer B (100mM potassium phosphate buffer containing 0.03mM of NaCl; the linear gradient started at 100% Buffer A to increase to 50% Buffer B over a 100 minute period, flow rate was 2ml/minute) to fraction recover the fractions eluting at a Buffer B concentration of from 4% to 6%. Further, the recovered fractions were subjected to a PD10 column (Amersham Pharmacia Biotech Company) and eluted with 0.05M potassium phosphate buffer (pH7.0) to recover the fractions containing protein.

A similar amount of 0.05M potassium phosphate buffer (pH7.0) containing 2.0M ammonium sulfate was added and mixed into the recovered fractions. The recovered fractions were then injected into a 1ml RESOURSE PHE column (Amersham Pharmacia Biotech Company). After flowing 5ml of 0.05M potassium phosphate buffer (pH7.0) containing 1M ammonium sulfate, the 0.05M potassium phosphate buffer (pH7.0) was flown with a linear gradient of ammonium sulfate (gradient of the ammonium sulfate concentration was 0.1M/minute, range of NaCl concentration was 1M to 0M, flow rate was 2ml/minute) to fraction recover the fractions eluting at an ammonium sulfate concentration of from about 0.4M to 0.5M. The protein contained in each of the fractions were analyzed on a 10%-20% SDS-PAGE.

Instead of the crude cell extract in the reaction solutions described in Example

25(2), the recovered fractions were added and maintained in the presence of component A, component B, component C and compound (II) labeled with ^{14}C, similarly to Example 25(2). The reaction solutions after the maintenance were TLC analyzed to examine the intensity of the spots corresponding to compound (III) labeled with ^{14}C. Said protein moving to a location of about 45kDa in the above SDS-PAGE was recovered from the gel and was subjected to an amino acid sequence analysis with a protein sequencer (Applied Biosystems Company, Procise 494HT, pulsed liquid method) to sequence the N terminus amino acid sequence. As a result, the amino acid sequence shown in SEQ ID NO: 113 was provided.

Example 26 Obtaining the Present Invention DNA (A4)

(1) Preparation of the chromosomal DNA of Streptomyces achromogenes IFO 12735

Streptomyces achromogenes IFO 12735 cultured with shaking at 30℃ for 1 day to 3 days in 50ml of YEME medium (0.3%(w/v) yeast extract, 0.5%(w/v) bacto-peptone, 0.3%(w/v) malt extract, 1.0%(w/v) glucose, 34%(w/v) sucrose and 0.2%(v/v) 2.5M $MgCl_2 \cdot 6H_2O$). The cells were recovered. The obtained cells were suspended in YEME medium containing 1.4%(w/v) glycine and 60mM EDTA and further incubated with shaking for a day. The cells were recovered from the culture medium. After washing once with distilled water, it was resuspended in buffer (100mM Tris-HCl (pH8.0), 100mM EDTA, 10mM NaCl) at 1ml per 200mg of the cells. Two hundred micrograms per milliliter (200μg/ml) of egg-white lysozyme were added. The cell suspension was shaken at 30℃ for a hour. Further, 0.5% of SDS and 1mg/ml of Proteinase K was added. The cell suspension was incubated at 55℃ for 3 hours. The cell suspension was extracted twice with phenol·chloroform·isoamyl alcohol to recover each of the aqueous

layers. Next, there was one extraction with chloroform-isoamyl alcohol to recover the aqueous layer. The chromosomal DNA was obtained by ethanol precipitating the aqueous layer.

(2) Preparation of the chromosomal DNA library of Streptomyces achromogenes IFO 12735

Thirty-eight micrograms (38μg) of the chromosomal DNA prepared in Example 26(1) were digested with 3.2U of restriction enzyme Sau3A1 at 37℃ for 60 minutes. The obtained digestion solution was separated with 1% agarose gel electrophoresis. The DNA of about 2.0kbp was recovered from the gel. The DNA was purified with QIAquick Gel Extraction Kit (Qiagen Company) according to the instructions attached to said kit and was concentrated with an ethanol precipitation to obtain 20μl of the solution containing the target DNA. Eight microliters (8μl) of the DNA solution, 100ng of plasmid vector pUC118 digested with restriction enzyme BamHI and treated with dephosphorylation and 16μl of the I solution from Ligation Kit Ver. 2 (Takara Shuzo Company) were mixed and maintained for 3 hours at 16℃. E coli DH5 α were transformed utilizing the ligation solution and were spread onto LB agar medium containing 50mg/l of ampicillin to culture overnight at 37℃. The obtained colonies were recovered from an agar medium. The plasmid was extracted. The obtained plasmids were designated as the chromosomal DNA library.

(3) Isolation of the present invention DNA (A4)

PCR was conducted by utilizing the chromosomal DNA prepared in Example 26(2) as the template. As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 114 and an oligonucleotide having

the nucleotide sequence shown in SEQ ID NO: 57. The nucleotide sequence shown in SEQ ID NO: 114 was designed based on the amino acid sequence shown in SEQ ID NO: 113. The Expand HiFi PCR System (Boehringer Manheim Company) was utilized to prepare the reaction solution. The PCR reaction solution amounted to 25μl by adding 2.5μl of the above chromosomal DNA library, the 2 primers each amounting to 7.5pmol, 0.2μl of dNTP mix (a mixture of 2mM of each of the 4 types of dNTP), 0.2μl of 10x buffer (containing $MgCl_2$), 0.38μl of Expand HiFi enzyme mix and distilled water. The reaction conditions of the PCR were after maintaining 97℃ for 2 minute, repeating 10 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 65℃ for 30 seconds and followed by 72℃ for 1 minute; then conducting 15 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 65℃ for 30 seconds and followed by 72℃ for 1 minute (wherein 20 seconds was added to the maintenance at 72℃ for each cycle); and then maintaining 72℃ for 7 minutes. After the maintenance, 2.5μl of the reaction solution was utilized as a template solution for conducting PCR for a second time. As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 115 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 57. The nucleotide sequence shown in SEQ ID NO: 115 was designed based on the amino acid sequence shown in SEQ ID NO: 113. Similar to the above method, the Expand HiFi PCR System (Boehringer Manheim Company) was utilized to conduct PCR. The reaction solution after the maintenance was subjected to 2% agarose gel electrophoresis. The gel area containing the DNA of about 800bp was recovered. The DNA was purified from the recovered gel by utilizing QIA quick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA was ligated to the TA cloning vector pCRII-TOPO (Invitrogen Company) according to the instructions attached to said vector and was introduced into E. Coli

TOP10F'. The plasmid DNA was prepared from the obtained E. coli transformant, utilizing Qiagen Tip20 (Qiagen Company). A sequencing reaction was conducted with Big Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing a primers having the nucleotide sequence shown in SEQ ID NO: 67 and a primer having the nucleotide sequence shown in SEQ ID NO: 68. The obtained plasmid was utilized as a template in the sequencing reaction. The reaction products were analyzed with a DNA sequencer 3100 (Applied Biosystems Japan Company). As a result, the nucleotide sequence shown in nucleotides 57 to 832 of the nucleotide sequence shown in SEQ ID NO: 110 was provided. In the provided nucleotide sequence, nucleotides 58-60 of the nucleotide sequence shown in SEQ ID NO: 110 encoded amino acid 20 in the amino acid sequence shown in SEQ ID NO: 113.

Next, PCR was conducted with the Expand HiFi PCR System (Boehringer Manheim Company) under the above-described conditions, utilizing as a template the chromosomal DNA library prepared in Example 26(2). As the primers, there was utilized a primer pairing of the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 116 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 59. The amplified DNA of about 1.4kbp was cloned into the cloning vector pCRII-TOPO. The plasmid DNA was prepared from the obtained E. coli transformants, utilizing Qiagen Tip20 (Qiagen Company). A sequencing reaction was conducted with Big Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing a primer having the nucleotide sequence shown in SEQ ID NO: 67 and a primer having the nucleotide sequence shown in SEQ ID NO: 68. The obtained plasmid was utilized as a template in the sequencing reaction. The reaction products were analyzed with a DNA sequencer 3100

(Applied Biosystems Japan Company). As a result, the nucleotide sequence shown in nucleotides 1 to 58 in the nucleotide sequence shown in SEQ ID NO: 110 was provided.

The cloning of the DNA elongating downstream from the 3' terminus of the nucleotide shown as nucleotide 832 of the nucleotide sequence shown in SEQ ID NO: 110 was conducted. Specifically, 13μg of the chromosomal DNA of Streptomyces achromogenes IFO 12735 prepared in Example 26(1) was digested overnight with 200U of restriction enzyme HincII at 37℃. After a phenol extraction, the DNA was purified by an ethanol precipitation. The obtained DNA was used to produce 20μl of an aqueous solution. Four microliters (4μl) thereof, 1.9μl of 15μM Genome Walker Adaptor, 1.6μl of 10x ligation buffer and 0.5μl of 6U/μl T4 ligase were mixed and maintained overnight at 16℃. After that, there was a maintenance at 70℃ for 5 minutes and an addition of 72μl of distilled water to provide a Genome Walker library. PCR was conducted by utilizing said library as a template. A PCR reaction solution amounting to 50μl was provided by adding 1μl of Genome Walker library and primer AP1 (provided with Universal Genome Walker Kit) and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 117 to each amount to 200nM, adding 1μl of dNTP mix (a mixture of 10mM each of the 4 types of dNTPs), 10μl of 5xGC genomic PCR buffer, 2.2μl of 25mM $Mg(OAc)_2$, 10μl of 5M GC-Melt and 1μl of Advantage-GC genomic polymerase mix and adding distilled water. The reaction conditions of the PCR were after maintaining 95℃ for 1 minute; conducting 7 cycles of a cycle that included maintaining 94℃ for 10 seconds and then 72℃ for 3 minutes; 36 cycles of a cycle that included maintaining 94℃ for 10 seconds and then 68℃ for 3 minutes; and maintaining 68℃ for 7 minutes. The reaction solution after the maintenance was diluted 50 fold with distilled water. The PCR products were designated as the first PCR products and were utilized as a template to conduct another PCR. The PCR amounting 50μl was provided by adding 1μl of the first PCR products and primer

AP2 (provided with Universal Genome Walker Kit) and the oligonucleotide shown in SEQ ID NO: 118 to each amount to 200nM, adding 1μl of dNTP mix (a mixture of 10mM each of the 4 types of dNTPs), 10μl of 5xGC genomic PCR buffer, 2.2μl of 25mM $Mg(OAc)_2$, 10μl of 5M GC-Melt and 1μl of Advantage-GC genomic polymerase mix and adding distilled water. The reaction conditions of the PCR were after maintaining 95℃ for 1 minute; conducting 5 cycles of a cycle that included maintaining 94℃ for 10 seconds and then 72℃ for 3 minutes; 20 cycles of a cycle that included maintaining 94℃ for 10 seconds and then 68℃ for 3 minutes; and maintaining 68℃ for 7 minutes. The reaction solution after the maintenance was subjected to 1% agarose gel electrophoresis. The gel area containing the DNA of about 1300bp was recovered. The DNA was purified from the recovered gel by utilizing QIA quick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA was ligated to cloning vector pCRII-TOPO (Invitrogen Company) according to the instructions attached to said vector and was introduced into E. Coli TOP10F'. The plasmid DNA was prepared from the E. coli transformant by utilizing Qiagen Tip20 (Qiagen Company). A sequencing reaction was conducted with Big Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the oligonucleotide shown in SEQ ID NO: 67 and the oligonucleotide shown in SEQ ID NO: 68. The obtained plasmid was utilized as a template in the sequencing reaction. The reaction products were analyzed with a DNA sequencer 3100 (Applied Biosystems Japan Company). As a result, the nucleotide sequence shown in nucleotides 644 to 1454 in the nucleotide sequence shown in SEQ ID NO: 110 was provided. As a result of connecting all of the analyzed nucleotide sequences, the nucleotide sequence shown in SEQ ID No: 110 was provided. Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 109) consisting of 1236

nucleotides (inclusive of the stop codon) and encoding a 411 amino acid residue (SEQ ID NO: 108) and a nucleotide sequence (SEQ ID NO: 112) consisting of 192 nucleotides (inclusive of the stop codon) and encoding a 63 amino acid residue (SEQ ID NO: 111). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 108) encoded by the nucleotide sequence shown in SEQ ID NO: 109 was calculated to be 45465Da. Further, the amino acid sequence encoded by said nucleotide sequence contained the amino acid sequence (SEQ ID NO: 113) determined from the amino acid sequencing of from the N terminus of the present invention protein (A4). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 111) encoded by the nucleotide sequence shown in SEQ ID NO: 112 was calculated to be 6871Da.

Example 27 The Expression of the Present Invention Protein (A4) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA(A4)

PCR was conducted by utilizing as a template the chromosomal DNA prepared from Streptomyces achromogenes IFO 12735 in Example 26(1) and by utilizing Expand HiFi PCR System (Boehringer Manheim Company). As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 119 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 120 (hereinafter referred to as "primer pairing 25") or a pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 119 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 121 (hereinafter referred to as "primer pairing 26"). The PCR reaction solution amounted to 50µl by adding the 2 primers each amounting to 300nM, 50ng of the above chromosomal DNA, 5.0µl of dNTP mix (a mixture of 2.0mM of each of the 4 types of dNTP), 5.0µl of 10x Expand HF buffer (containing $MgCl_2$) and 0.75µl of Expand HiFi enzyme mix and distilled water. The

reaction conditions of the PCR were after maintaining 97℃ for 2 minutes; repeating 10 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 1 minute; then conducting 15 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 1 minute (wherein 20 seconds was added to the maintenance at 72℃ for each cycle); and then maintaining 72℃ for 7 minutes. After the maintenance, the reaction solution was subjected to 1% agarose gel electrophoresis. The gel area containing the DNA of about 1.3kbp was recovered from the gel which was subjected the reaction solution utilizing primer pairing 25. The gel area containing the DNA of about 1.6kbp was recovered from the gel which was subjected the reaction solution utilizing primer pairing 26. The DNA were purified from each of the recovered gels by utilizing QIA quick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA were ligated to the cloning vector pCRII-TOPO (Invitrogen Company) according to the instructions attached to said vector and were introduced into E. Coli TOP10F'. The plasmid DNA were prepared from the obtained E. coli transformants, utilizing Qiagen Tip20 (Qiagen Company). Next, sequencing reactions were conducted with Big Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the oligonucleotides shown in SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 122 and SEQ ID NO: 123. The sequencing reactions utilized the obtained plasmid DNA as the template. The reaction products were analyzed with a DNA sequencer 3100 (Applied Biosystems Japan Company). Based on the results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 109 was designated as pCR646 and the plasmid having the nucleotide sequence shown in SEQ ID NO: 110 was designated as pCR646F.

Next, each of plasmids pCR646 and pCR646F was digested with restriction

enzymes NdeI and HindIII. The digestion products were subjected to agarose gel electrophoresis. The gel area containing a DNA of about 1.3kbp was cut from the gel subjected to the digestion products of pCR646. The gel area containing a DNA of about 1.6kbp was cut from the gel subjected to the digestion products of pCR646F. The DNA were purified from each of the recovered gels by utilizing QIA quick gel extraction kit (Qiagen Company) according to the attached instructions. Each of the obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated with ligation kit Ver.1 (Takara Shuzo Company) according to the instructions attached to said kit and introduced into E. Coli JM109. The plasmid DNA were prepared from the obtained E. coli transformants. The structures thereof were analyzed. The plasmid containing the nucleotide sequence shown in SEQ ID NO: 109, in which the DNA of about 1.3kbp encoding the present invention protein (A4) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN646. Further, the plasmid containing the nucleotide sequence shown in SEQ ID NO: 110, in which the DNA of about 1.6kbp encoding the present invention protein (A4) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN646F. Each of the above plasmids of pKSN646 and pKSN646F was introduced into E. coli JM109. The obtained E. coli transformants were designated, respectively, JM109/pKSN646 and JM109/pKSN646F. Further, plasmid pKSN2 was introduced into E. coli JM109. The obtained E. coli transformant was designated as JM109/pKSN2.

(2) Expression of the present invention protein (A4) in E. coli and recovery of said protein

E. coli JM109/pKSN646, JM109/pKSN646F and JM109/pKSN2 are each cultured overnight at 37℃ in 10ml of TB medium (1.2%(w/v) tryptone, 2.4%(w/v) yeast

extract, 0.4%(w/v) glycerol, 17mM potassium dihydrogenphosphate, 72mM dipotassium hydrogenphosphate) containing 50μg/ml of ampicillin. A milliliter (1ml) of the obtained culture medium is transferred to 100ml of TB medium containing 50μg/ml of ampicillin and cultured at 26℃. When OD660 reaches about 0.5, 5-aminolevulinic acid is added to the final concentration of 500μM, and the culturing is continued. Thirty (30) minutes thereafter, IPTG is added to a final concentration of 1mM, and there is further culturing for 17 hours.

The cells are recovered from each of the culture mediums, washed with 0.1M tris-HCl buffer (pH7.5) and suspended in 10 ml of the above buffer containing 1mM PMSF. The obtained cell suspensions are subjected 6 times to a sonicator (Sonifier (Branson Sonic Power Company)) at 3 minutes each under the conditions of output 3, duty cycle 30%, in order to obtain cell lysate solutions. After centrifuging the cell lysate solutions (1,200xg, 5 minutes) the supernatants are recovered and centrifuged (150,000xg, 70 minutes) to recover supernatant fractions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN646 is referred to as "E. coli pKSN646 extract ", the supernatant fraction obtained from E. coli JM109/pKSN646F is referred to as "E. coli pKSN646F extract", and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract "). A microliter (1μl) of the above supernatant fractions is analyzed on a 15% to 25% SDS-PAGE and stained with CBB. As a result, by detecting notably more intense bands in both E. coli pKSN646 extract and E. coli pKSN646F extract than the E. coli pKSN2 extract, at the electrophoresis locations corresponding to the molecular weight of 45kDa, it can be confirmed that the present invention protein (A4) is expressed in E. coli.

(3) Detection of the ability to convert compound (II) to compound (III)

Reaction solutions of 30μl are prepared and maintained for 10 minutes at 30℃. The reaction solutions consist of a 0.1M potassium phosphate buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2mM of β -NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 2mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 0.1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 18μl of the supernatant fraction recovered in Example 27(2). Further, there are prepared and maintained similarly reaction solutions having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B and component C. Three microliters (3μl) of 2N HCl and 90 μl of ethyl acetate are added and mixed into each of the reaction solutions after the maintenance. The resulting reaction solutions are centrifuged at 8,000xg to recover 75μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue is dissolved in 6.0μl of ethyl acetate. Five microliters (5.0μl) thereof is spotted to a silica gel TLC plate (TLC plate silica gel $60F_{254}$, 20cm x 20cm, 0.25mm thick, Merck Company). The TLC plate is developed with a 6: 1: 2 mixture of chloroform, acetic acid and ethyl acetate for about 1 hour. The solvents are then allowed to evaporate. The TLC plate is exposed overnight to an imaging plate (Fuji Film Company). Next, the imaging plate was analyzed on Image Analyzer BAS2000 (Fuji Film Company). The presence of a spot corresponding to compound (III) labeled with ^{14}C is examined (Rf value 0.24 and 0.29). The production of compound (III) in reaction solutions containing component A, component B, component C and E. coli pKSN646 extract, or in reaction solutions containing component A, component B, component C and E. coli pKSN646F extract can be confirmed.

Example 28 Sequence Identity Relating to the Present Invention Protein

The sequence identity relating to the proteins of the present invention and the DNA of the present invention was analyzed by utilizing GENETYX-WIN Ver. 5 (Software Development Company). The alignments were produced by conducting the homology analysis with the Lipman-Pearson method (Lipman, D.J. and Pearson, W.R., Science, 227, 1435-1441, (1985)).

In regards to amino acid sequences of the present invention proteins (A1) to (A4), there were determined the sequence identities to each other and to known proteins of the highest homology. The results are shown in Table 15.

Table 15

	present invention protein (A1)	present invention protein (A2)	present invention protein (A3)	present invention protein (A4)	known proteins of the highest homology*
present invention protein (A1)	100%	47%	64%	48%	73% AAC25766
present invention protein (A2)	47%	100%	48%	51%	52% CAB46536
present invention protein (A3)	64%	48%	100%	46%	67% AAC25766
present invention protein (A4)	48%	51%	46%	100%	50% CAB46536

*the sequence identity is shown on top and the accession number of the provided protein in the Entrez database (provided by Center for Biotechnology Information, http://www3.ncbi.nlm.nih.gov/Entrez/) is shown on the bottom.

In regards to the nucleotide sequences of the present invention DNA (A1) having

the nucleotide sequence shown in SEQ ID NO: 6, the present invention DNA (A2) having the nucleotide sequence shown in SEQ ID NO: 7, the present invention DNA (A3) having the nucleotide sequence shown in SEQ ID NO: 8 and the present invention DNA (A4) having the nucleotide sequence shown in SEQ ID NO: 109, there were determined the sequence identities to each other and to known genes of the highest homology. The results are shown in Table 16.

Table 16

	SEQ ID NO: 6 [present invention DNA (A1)]	SEQ ID NO: 7 [present invention DNA (A2)]	SEQ ID NO: 8 [present invention DNA (A3)]	SEQ ID NO: 109 [present invention DNA (A4)]	known genes of the highest homology*
SEQ ID NO: 6 [present invention DNA (A1)]	100%	61%	74%	62%	77% AF072709
SEQ ID NO: 7 [present invention DNA (A2)]	61%	100%	64%	65%	66% Y18574
SEQ ID NO: 8 [present invention DNA (A3)]	74%	64%	100%	63%	75% AF072709
SEQ ID NO: 109 [present invention DNA (A4)]	62%	65%	63%	100%	64% Y18574

*the sequence identity is shown on top and the accession number of the provided gene in the Entrez database (provided by Center for Biotechnology Information, http://www3.ncbi.nlm.nih.gov/Entrez/) is shown on the bottom.

In regards to the amino acid sequences of the present invention proteins (B1) to (B4), there were determined the sequence identities to each other and to known proteins of the highest homology. The results are shown in Table 17.

Table 17

	present invention protein (B1)	present invention protein (B2)	present invention protein (B3)	present invention protein (B4)	known proteins of the highest homology*
present invention protein (B1)	100%	45%	78%	41%	76% AAC25765
present invention protein (B2)	45%	100%	40%	41%	60% AAF71770
present invention protein (B3)	78%	40%	100%	40%	73% AAC25765
present invention protein (B4)	41%	41%	40%	100%	55% AAA26824

*the sequence identity is shown on top and the accession number of the provided protein in the Entrez database (provided by Center for Biotechnology Information, http://www3.ncbi.nlm.nih.gov/Entrez/) is shown on the bottom.

In regards to the nucleotide sequences of the present invention DNA (B1) having the nucleotide sequence shown in SEQ ID NO: 15, the present invention DNA (B2) having the nucleotide sequence shown in SEQ ID NO: 16, the present invention DNA (B3) having the nucleotide sequence shown in SEQ ID NO: 17 and the present invention DNA (B4) having the nucleotide sequence shown in SEQ ID NO: 112, there were determined the sequence identities to each other and to known genes of the highest homology. The results are shown in Table 18.

Table 18

	SEQ ID NO: 15 [present invention DNA (B1)]	SEQ ID NO: 16 [present invention DNA (B2)]	SEQ ID NO: 17 [present invention DNA (B3)]	SEQ ID NO: 112 [present invention DNA (B4)]	known genes of the highest homology*
SEQ ID NO: 15 [present invention DNA (B1)]	100%	60%	80%	59%	84% AF072709
SEQ ID NO: 16 [present invention DNA (B2)]	60%	100%	60%	59%	66% M32238
SEQ ID NO: 17 [present invention DNA (B3)]	80%	60%	100%	65%	79% AF072709
SEQ ID NO: 112 [present invention DNA (B4)]	59%	59%	65%	100%	66% M32239

*the sequence identity is shown on top and the accession number of the provided gene in the Entrez database (provided by Center for Biotechnology Information, http://www3.ncbi.nlm.nih.gov/Entrez/) is shown on the bottom.

Example 29 PCR Utilizing an Oligonucleotide Having a Partial Nucleotide Sequence of the Present Invention DNA (A) as a Primer

PCR was conducted by utilizing as a template each of: the chromosomal DNA of Streptomyces phaeochromogenes IFO 12898 prepared in Example 2; the chromosomal DNA of Saccharopolyspora taberi JCM 9383t prepared in Example 5; the chromosomal DNA of Streptomyces griseolus ATCC 11796 prepared in Example 9; the chromosomal DNA of Streptomyces testaceus ATCC 21469 prepared in Example 11; the chromosomal DNA of Streptomyces achromogenes IFO 12735 prepared in Example 26; and each of the chromosomal DNA of Streptomyces griseofuscus IFO 12870t, Streptomyces

thermocoerulescens IFO 14273t and Streptomyces nogalater IFO 13445 prepared similarly to the method described in Example 2. As the primers, the 5 pairings of primers shown in Table 19 were utilized. The predicted size of the DNA amplified by the PCR utilizing each of the primer pairings based on the nucleotide sequence shown in SEQ ID NO: 6 is shown in Table 19.

Table 19

primer pairing	primer	primer	amplified DNA
14	SEQ ID NO: 124	SEQ ID NO: 129	about 800bp
15	SEQ ID NO: 125	SEQ ID NO: 129	about 600bp
16	SEQ ID NO: 126	SEQ ID NO: 129	about 600bp
17	SEQ ID NO: 127	SEQ ID NO: 129	about 580bp
18	SEQ ID NO: 128	SEQ ID NO: 129	about 580bp

The PCR reaction solution amounted to 25μl by adding 200nM of each of the 2 primers of the pairing shown in Table 19, adding 10ng of the chromosomal DNA, 0.5μl of dNTP mix (a mixture of 10mM of each of the 4 types of dNTP), 5μl of 5xGC genomic PCR buffer, 1.1μl of 25mM $Mg(OAc)_2$, 5μl of 5M GC-Melt and 0.5μl of Advantage-GC genomic polymerase mix and adding water. The reaction conditions were maintaining 95℃ for 1 minute; repeating 30 cycles of a cycle that included maintaining 94℃ for 15 seconds, followed by 60℃ for 30 seconds, and followed by 72℃ for 1 minute; and maintaining 72℃ for 5 minutes. Each of the reaction solutions after the maintenance was analyzed with 3% agarose gel electrophoresis. The results are shown in Fig. 46 and in Table 20 and Table 21. The amplification of the predicted size of the DNA was observed in each or all of the cases with primer pairings 14, 15, 16, 17 and 18 as well as in the cases of utilizing the chromosomal DNA prepared from any of the strains as a template.

Table 20

Lane	Reagents		amplification of DNA*
	origin of the template chromosomal DNA	primer pairing	
2	Streptomyces phaeochromogenes IFO 12898	14	+
3	Streptomyces phaeochromogenes IFO 12898	15	+
4	Streptomyces phaeochromogenes IFO 12898	16	+
5	Streptomyces phaeochromogenes IFO 12898	17	+
6	Streptomyces phaeochromogenes IFO 12898	18	+
9	Streptomyces testaceus ATCC 21469	14	+
10	Saccharopolyspora taberi JCM 9393t	14	+
11	Streptomyces griseolus ATCC 11796	14	+
13	Streptomyces testaceus ATCC 21469	15	+
14	Saccharopolyspora taberi JCM 9393t	15	+
15	Streptomyces griseolus ATCC 11796	15	+
16	Streptomyces testaceus ATCC 21469	16	+
17	Saccharopolyspora taberi JCM 9393t	16	+
18	Streptomyces griseolus ATCC 11796	16	+
20	Streptomyces testaceus ATCC 21469	17	+
21	Saccharopolyspora taberi JCM 9393t	17	+
22	Streptomyces griseolus ATCC 11796	17	+
23	Streptomyces testaceus ATCC 21469	18	+
24	Saccharopolyspora taberi JCM 9393t	18	+
25	Streptomyces griseolus ATCC 11796	18	+

* "+" represents that the predicted size of the DNA was detected and "-" represents that there was no detection.

Table 21

Lane	Reagents		amplification of DNA*
	Origin of template chromosomal DNA	primer pairing	
28	Streptomyces griseofuscus IFO 12870t	14	+
29	Streptomyces thermocoerulescens IFO 14273t	14	+
30	Streptomyces achromogenes IFO 12735	14	−
31	Streptomyces nogalater IFO 13445	14	+
33	Streptomyces griseofuscus IFO 12870t	15	+
34	Streptomyces thermocoerulescens IFO 14273t	15	+
35	Streptomyces achromogenes IFO 12735	15	−
36	Streptomyces nogalater IFO 13445	15	+
38	Streptomyces griseofuscus IFO 12870t	16	+
39	Streptomyces thermocoerulescens IFO 14273t	16	+
40	Streptomyces achromogenes IFO 12735	16	+
41	Streptomyces nogalater IFO 13445	16	+
43	Streptomyces griseofuscus IFO 12870t	17	+
44	Streptomyces thermocoerulescens IFO 14273t	17	+
45	Streptomyces achromogenes IFO 12735	17	+
46	Streptomyces nogalater IFO 13445	17	+
48	Streptomyces griseofuscus IFO 12870t	18	−
49	Streptomyces thermocoerulescens IFO 14273t	18	+
50	Streptomyces achromogenes IFO 12735	18	−
51	Streptomyces nogalater IFO 13445	18	+

* "+" represents that the predicted size of the DNA was detection and "-" represents that there was no detection.

Example 30 Hybridization Utilizing as a Probe a DNA Consisting of a Partial Nucleotide Sequence of the Present DNA (A) and the Present Invention DNA (A)

(1) Preparation of a Probe

DNA consisting of a partial nucleotide sequence of the present invention DNA

(A1) or a partial nucleotide sequence of the present invention DNA (A1) was produced as a probe labeled with digoxigenin (DIG labeled probe). PCR was conducted with PCR DIG Probe synthesis kit (Roche Diagnostics GmbH Company) according to the attached manual by utilizing as a template the chromosomal DNA of Streptomyces phaeochromogenes IFO 12898 prepared in Example 3 and by utilizing as primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 93 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 94. The PCR reaction solution amounted to 50μl by adding the 2 primers each amounting to 200nM, adding 50ng of the chromosomal DNA, 2.5μl of dNTP mix (a mixture of 2.0mM of each of the 4 types of dNTP), 2.5μl of PCR DIG mix (a mixture of 2.0mM of each of the 4 types of dNTP labeled with DIG), 5μl of 10x PCR buffer and 0.75μl of Expand HiFi enzyme mix and adding distilled water. The reaction conditions were after maintaining 95℃ for 2 minutes; repeating 10 cycles of a cycle that included maintaining 95℃ for 10 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 2 minutes; then conducting 15 cycles of a cycle that included maintaining 95℃ for 10 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 2 minutes (wherein 20 seconds was added to the maintenance at 72℃ for each cycle); and then maintaining 72℃ for 7 minutes. The reaction solution after the maintenance was subjected to 1% agarose gel electrophoresis. As a result, amplification of a DNA of about 1.3kb was confirmed. The amplified DNA was recovered to obtain a DNA labeled with digoxigenin and having the nucleotide sequence shown in SEQ ID NO: 6. Under a similar method, PCR was conducted by utilizing as a template the chromosomal DNA of Streptomyces phaeochromogenes IFO 12898 and by utilizing as the primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 130 and the oligonucleotide consisting of the nucleotide sequence show in SEQ ID NO: 131. The DNA amplified by

said PCR was recovered to obtain a DNA labeled with digoxigenin and having the nucleotide sequence shown in nucleotides 57 to 730 of the nucleotide sequence shown in SEQ ID NO: 6.

Under a similar method, PCR was conducted by utilizing as a template the chromosomal DNA of Saccharopolyspora taberi JCM 9393t prepared in Example 6 and by utilizing as primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 61 and the oligonucleotide sequence consisting of the nucleotide sequence shown in SEQ ID NO: 62. The DNA amplified by said PCR was recovered to obtain a DNA labeled with digoxigenin and having the nucleotide sequence shown in SEQ ID NO: 7.

Further, under a similar method, PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces testaceus ATCC 21469 prepared in Example 11 and by utilizing as primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 70 and the oligonucleotide sequence consisting of the nucleotide sequence shown in SEQ ID NO: 71. The DNA amplified by said PCR was recovered to obtain a DNA labeled with digoxigenin and having the nucleotide sequence shown in SEQ ID NO: 8. Further, PCR was conducted by utilizing the above-mentioned chromosomal DNA as the template and by utilizing as the primers the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 132 and the oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 133. The DNA amplified by said PCR was recovered to obtain a DNA labeled with digoxigenin and having the nucleotide sequence shown in nucleotides 21 to 691 of the nucleotide sequence shown in SEQ ID NO: 8.

(2) Dot-blot Hybridization

Each of the DNA of pKSN657 prepared in Example 4 (the DNA comprising the present invention DNA (A1)), the DNA of pKSN923 prepared in Example 7 (the DNA comprising the present invention DNA (A2)), the DNA of pKSN671 prepared in Example 12 (the DNA comprising the present invention DNA (A3)), the DNA of pKSNSCA prepared in Example 14 (the DNA comprising the present DNA (A9)) and the DNA of pKSN11796 prepared in Example 10 (the DNA comprising the present DNA (A10)) was blotted onto a nylon membrane Hybond N+ (Amersham Pharmacia Company) to amount to 100ng and 10ng. Ultraviolet light was directed at the obtained membranes with a transilluminator for 5 minutes.

DIG-High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics GmbH Company) was utilized for the hybridization and detection according to the attached manual. As the probes, each of the DNA labeled with digoxigenin and produced in Example 30(1) which were maintained at 100℃ for 5 minutes and then quickly cooled in ice (hereinafter, referred to as "DIG labeled probe") was utilized. The dotted above membrane was shaken at 42℃ for 30 minutes in 2.0ml of DIGEasyHyb that was provided with said kit. Next, 2.0ml of Dig Easy Hyb, 5.0µl of the DIG labeled probes and the membrane were enclosed in a plastic bag for hybridization and maintained at 42℃ for 18 hours. The membrane was recovered, was shaken twice in 2x SSC containing 0.1% SDS for 5 minutes at room temperature and was then shaken twice in 0.5xSSC containing 0.1%SDS at 65℃ for 15 minutes. Subsequently, the membrane was shaken in 50ml of washing buffer for 2 minutes, then shaken in 50ml of blocking solution at room temperature for 30 minutes, then shaken in 2.0ml of antibody solution for 30 minutes, and then shaken twice in 50ml of washing buffer for 15 minutes. Further, after shaking in 50ml of detection buffer for 5 minutes, the membrane was enclosed in a hybridization bag with 2.0ml of Color Substrate solution and maintained at room

temperature for 18 hours. A signal was detected in each of the cases of conducting hybridization with each of the reagents of 10ng and 100ng of each of pKSN657, pKSN923, pKSN671, pKSNSCA and pKSN11796.

Example 31 Obtaining the Present Invention DNA (A11)

(1) Preparation of the chromosomal DNA of Streptomyces nogalator IFO13445

Streptomyces nogalator IFO13445 was cultivated with shaking at 30℃ for 3 days in 50ml of YGY medium (0.5%(w/v) yeast extract, 0.5%(w/v) tryptone, 0.1%(w/v) glucose and 0.1%(w/v) K_2HPO_4, pH7.0). The cells were recovered. The obtained cells were suspended in YGY medium containing 1.4%(w/v) glycine and 60mM EDTA and further incubated with shaking for a day. The cells were recovered from the culture medium. After washing once with distilled water, it was suspended in 3.5ml of Buffer B1 (50mM Tris-HCl (pH8.0), 50mM EDTA, 0.5% of Tween-20 and 0.5% Triton X-100). Eighty microliters (80μl) of a 100μg/ml lysozyme solution and 100μl of Qiagen Protease (600mAU/ml, Qiagen Company) were added to the suspension and maintained at 37℃ for a hour. Next, 1.2ml of Buffer B2 (3M guanidine HCl and 20% tween-20) was added, mixed and maintained at 50℃ for 30 minutes. The obtained cell lysate solution added to a Qiagen genomic chip 100G (Qiagen Company) equalized in Buffer QBT (750mM NaCl, 50mM MOPS (pH7.0), 15% isopropanol and 0.15% Triton X-100). Next, after the chip was washed twice with 7.5ml of Buffer QC (50mM MOPS (pH7.0) and 15% isopropanol), the DNA was eluted by flowing 5ml of Buffer QF (1.25M NaCl, 50mM Tris HCl (pH8.5), 15% isopropanol). Three and five-tenths milliliters (3.5ml) of isopropanol was mixed into the obtained DNA solution to precipitate and recover the chromosomal DNA. After washing with 70% ethanol, the recovered chromosomal DNA was dissolved in 1ml of TB buffer.

(2) **Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A11)**

PCR was conducted by utilizing as the template the chromosomal DNA prepared in Example 31(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. The amplified DNA was ligated to cloning vector pCRII-TOPO (Invitrogen Company) according to the instructions attached to said vector and was then introduced into E. Coli TOP10F'. The plasmid DNA was prepared from the obtained E. coli transformant, utilizing Qiagen Tip20 (Qiagen Company). A sequencing reaction was conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing a primer having the nucleotide sequence shown in SEQ ID NO: 57 and a primer having the nucleotide sequence shown in SEQ ID NO: 59. The sequence reaction utilized the obtained plasmid as a template. The reaction products were analyzed with a DNA sequencer 3100 (Applied Biosystems Japan Company). As a result, the nucleotide sequence shown in nucleotides 316 to 1048 of the nucleotide sequence shown in SEQ ID NO: 139 was provided.

Further, the chromosomal DNA prepared in Example 31(1) was digested with restriction enzyme PvuII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 161 and primer AP1 (Universal Genome Walker Kit (Clontech Company)). Next, PCR was conducted under the conditions decribed in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide

having the nucleotide sequence shown in SEQ ID NO: 162 and primer AP2 (Universal Genome Walker Kit (Clontech Company)). The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 330 of the nucleotide sequence shown in SEQ ID NO: 144 was provided.

Further, the chromosomal DNA prepared in Example 31(1) was digested with restriction enzyme HincII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 163 and primer AP1 (Universal Genome Walker Kit (Clontech Company)). Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 164 and primer AP2 (Universal Genome Walker Kit (Clontech Company)). The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 983 to 1449 of the nucleotide sequence shown in SEQ ID NO: 144 was provided.

(3) Sequence analysis of the present invention DNA (A11)

The nucleotide sequence shown in SEQ ID NO: 144 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 31(2). Two open reading frames (ORF) were present. As such, there was contained a nucleotide sequence (SEQ ID NO: 139) consisting of 1230 nucleotides (inclusive of the stop codon) and encoding a 409 amino acid residue (SEQ ID NO: 159) and a nucleotide sequence (SEQ ID NO: 154) consisting of 207 nucleotides (inclusive of the stop codon) and encoding a 68 amino acid residue (SEQ ID NO: 149). The molecular weight of the protein consisting of the amino

acid sequence (SEQ ID NO: 159) encoded by the nucleotide sequence shown in SEQ ID NO: 139 was calculated to be 45177Da. Further, the molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 149) encoded by the nucleotide sequence shown in SEQ ID NO: 154 was calculated to be 7147Da.

Example 32 Expression of the Present Invention Protein (A11) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA(A11)

PCR was conducted by utilizing as a template the chromosomal DNA prepared from Streptomyces nogalator IFO13445 in Example 31(1) and by utilizing Expand HiFi PCR System (Boehringer Manheim Company). As the primers, there was utilized the pairing of an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 165 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 166. The reaction solution composition and the maintenance were similar to the conditions described in Example 27(1). The reaction solution after the maintenance was subjected to 1% agarose gel electrophoresis. The gel area containing the DNA of about 1.5kbp was recovered. The DNA was purified from the recovered gel by utilizing QIA quick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA was ligated to the cloning vector pCRII-TOPO (Invitrogen Company) according to the instructions attached to said vector and was introduced into E. Coli TOP10F'. The plasmid DNA was prepared from the obtained E. coli transformants, utilizing Qiagen Tip20 (Qiagen Company). Sequencing reactions were conducted with Dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems Japan Company) according to the instructions attached to said kit, utilizing as primers the oligonucleotides having the nucleotide sequences shown in, respectively, SEQ ID NOs: 57, 59, and 186. The sequencing reactions utilized the obtained plasmid DNA as the template. The reaction

products were analyzed with a DNA sequencer 3100 (Applied Biosystems Japan Company). Based on the results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 144 was designated as pCR849AF.

Next, pCR849AF was digested with restriction enzymes NdeI and HindIII. The digestion products were subjected to agarose gel electrophoresis. The gel area containing a DNA of about 1.5kbp was cut from the gel. The DNA was purified from the recovered gels by utilizing QIA quick gel extraction kit (Qiagen Company) according to the attached instructions. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated with ligation kit Ver.2 (Takara Shuzo Company) according to the instructions attached to said kit and introduced into E. Coli JM109. The plasmid DNA were prepared from the obtained E. coli transformants. The structures thereof were analyzed. The plasmid containing the nucleotide sequence shown in SEQ ID NO: 144, in which the DNA of about 1.5kbp encoding the present invention protein (A11) is inserted between the NdeI site and the HindIII site of pKSN2 was designated as pKSN849AF. Plasmid pKSN849AF was introduced into E. coli JM109. The obtained E. coli transformant was designated JM109/pKSN849AF. Further, plasmid pKSN2 was introduced into E. coli JM109. The obtained E. coli transformant was designated as JM109/pKSN2.

(2) Expression of the present invention protein (A11) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN849AF and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained

from E. coli JM109/pKSN849AF is referred to as "E. coli pKSN849AF extract " and the supernatant fraction obtained from JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) **Detection of the ability to convert compound (II) to compound (III)**

Reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. The reaction solutions consisted of a 0.1M potassium phosphate buffer (pH7.0) containing 3ppm of compound (II) labeled with ^{14}C, 2mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 2mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 0.1U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 23μl of the supernatant fraction recovered in Example 32(2). Similarly to Example 4(3), the reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN849AF extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 33 Obtaining the Present Invention DNA (A12)

(1) **Preparation of the chromosomal DNA of Streptomyces tsusimaensis IFO 13782**

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces tsusimaensis IFO 13782 was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A12)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces tsusimaensis IFO 13782 prepared in Example 33(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 364 to 1096 of the nucleotide sequence shown in SEQ ID NO: 140 was provided.

Further, the chromosomal DNA prepared in Example 33(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 167 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 168 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 392 of the nucleotide sequence shown in SEQ ID NO: 145 was provided.

Further, the chromosomal DNA prepared in Example 33(1) was digested with restriction enzyme PvuII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide

sequence shown in SEQ ID NO: 169 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 170 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1048 to 1480 of the nucleotide sequence shown in SEQ ID NO: 145 was provided.

(3) Sequence analysis of the present invention DNA (A12)

The nucleotide sequence shown in SEQ ID NO: 145 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 33(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 140) consisting of 1278 nucleotides (inclusive of the stop codon) and encoding a 425 amino acid residue (SEQ ID NO: 160) and a nucleotide sequence (SEQ ID NO: 155) consisting of 198 nucleotides (inclusive of the stop codon) and encoding a 65 amino acid residue (SEQ ID NO: 150). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 160) encoded by the nucleotide sequence shown in SEQ ID NO: 140 was calculated to be 46549Da. Further, the molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 150) encoded by the nucleotide sequence shown in SEQ ID NO: 155 was calculated to be 6510Da.

Example 34 Expression of the Present Invention DNA (A12) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A12)

PCR was conducted similarly to Example 32(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces tsusimaensis IFO 13782 in Example 33(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence

shown in SEQ ID NO: 171 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 172. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was analyzed with oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 57, 59, 171, 172 and 187. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 145 was designated as pCR1618F. Similarly to Example 32(1), pCR1618F was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 145, in which the DNA encoding the present invention protein (A12) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN1618F"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN1618F.

(2) Expression of the present invention protein (A12) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN1618F and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN1618F is referred to as "E. coli pKSN1618F extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. Other than utilizing the supernatant fractions recovered in Example 34(2) (E. coli pKSN1618F extract or E. coli pKSN2 extract), the reaction solutions were prepared similarly to Example 32(3). The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN1618F extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 35 Obtaining the Present Invention DNA (A13)

(1) Preparation of the chromosomal DNA of Streptomyces thermocoerulesces IFO 14273t

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces thermocoerulesces IFO 14273t was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A13)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces thermocoerulesces IFO 14273t prepared in Example 35(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO

(Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 295 to 1027 of the nucleotide sequence shown in SEQ ID NO: 141 was provided.

Further, the chromosomal DNA prepared in Example 35(1) was digested with restriction enzyme HincII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 173 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 174 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 370 of the nucleotide sequence shown in SEQ ID NO: 146 was provided.

Further, the chromosomal DNA prepared in Example 35(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 175 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 176 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 960 to 1473 of the nucleotide sequence shown in SEQ ID NO: 146 was provided.

(3) Sequence analysis of the present invention DNA (A13)

The nucleotide sequence shown in SEQ ID NO: 146 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 35(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 141) consisting of 1209 nucleotides (inclusive of the stop codon) and encoding a 402 amino acid residue (SEQ ID NO: 136) and a nucleotide sequence (SEQ ID NO: 156) consisting of 252 nucleotides (inclusive of the stop codon) and encoding a 83 amino acid residue (SEQ ID NO: 151). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 136) encoded by the nucleotide sequence shown in SEQ ID NO: 141 was calculated to be 44629Da. Further, the molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 151) encoded by the nucleotide sequence shown in SEQ ID NO: 156 was calculated to be 8635Da.

Example 36 Expression of the Present Invention DNA (A13) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A13)

PCR was conducted similarly to Example 32(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces thermocoerulesces IFO 14273t in Example 35(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 177 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 178. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was analyzed with oligonucleotides having nucleotide sequences shown, respectively, in SEQ ID NOs: 57, 59, 173, 175 and 188. Based on the obtained results, the plasmid having the

nucleotide sequence shown in SEQ ID NO: 146 was designated as pCR474F. Similarly to Example 32(1), pCR474F was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 146, in which the DNA encoding the present invention protein (A13) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN474F"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN474F.

(2) Expression of the present invention protein (A13) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN474F and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN474F is referred to as "E. coli pKSN474F extract " and the supernatant fraction obtained from JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. Other than utilizing the supernatant fractions recovered in Example 36(2) (E. coli pKSN474F extract or E. coli pKSN2 extract), the reaction solutions were prepared similarly to Example 32(3). The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the

TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN474F extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 37 **Obtaining the Present Invention DNA (A14)**

(1) Preparation of the chromosomal DNA of Streptomyces thermocoerulesces IFO 14273t

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces glomerochromogenes IFO 13673t was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A13)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces glomerochromogenes IFO 13673t prepared in Example 37(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 316 to 1048 of the nucleotide sequence shown in SEQ ID NO: 142 was provided.

Further, the chromosomal DNA prepared in Example 37(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide

sequence shown in SEQ ID NO: 179 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 180 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 330 of the nucleotide sequence shown in SEQ ID NO: 147 was provided.

Further, the chromosomal DNA prepared in Example 37(1) was digested with restriction enzyme HincII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 181 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 182 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 982 to 1449 of the nucleotide sequence shown in SEQ ID NO: 147 was provided.

(3) Sequence analysis of the present invention DNA (A14)

The nucleotide sequence shown in SEQ ID NO: 147 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 37(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 142) consisting of 1230 nucleotides (inclusive of the stop codon) and encoding a 409 amino acid residue (SEQ ID NO: 137) and a nucleotide sequence (SEQ ID NO: 157) consisting of 207 nucleotides (inclusive of the stop codon) and

encoding a 68 amino acid residue (SEQ ID NO: 152). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 137) encoded by the nucleotide sequence shown in SEQ ID NO: 142 was calculated to be 45089Da. Further, the molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 152) encoded by the nucleotide sequence shown in SEQ ID NO: 157 was calculated to be 7174Da.

Example 38 Expression of the Present Invention DNA (A14) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A14)

PCR was conducted similarly to Example 32(1), other than utilizing as a template the chromosomal DNA of Streptomyces glomerochromogenes IFO 13673t prepared in Example 37(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 183 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 184. Similarly to Example 32(1), the DNA was purified from the PCR reaction solution and cloned into cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was analyzed with oligonucleotides having nucleotide sequences shown, respectively, in SEQ ID NOs: 57, 59 and 189. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 147 was designated as pCR1491AF. Similarly to Example 32(1), pCR1491AF was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 147, in which the DNA encoding the present invention protein (A14) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN1491AF"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN1491AF.

(2) Expression of the present invention protein (A14) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN1491AF and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN1491AF is referred to as "E. coli pKSN1491AF extract " and the supernatant fraction obtained from JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. Other than utilizing the supernatant fractions recovered in Example 38(2) (E. coli pKSN1491AF extract or E. coli pKSN2 extract), the reaction solutions were prepared similarly to Example 32(3). The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN1491AF extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 39 Obtaining the Present Invention DNA (A15)

(1) Preparation of the chromosomal DNA of Streptomyces olivochromogenes

IFO 12444

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces olivochromogenes IFO 12444 was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A15)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces olivochromogenes IFO 12444 prepared in Example 39(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 316 to 1048 of the nucleotide sequence shown in SEQ ID NO: 143 was provided.

Further, the chromosomal DNA prepared in Example 37(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained DNA as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 179 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 180 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 330 of the nucleotide sequence shown in SEQ ID NO: 148 was provided.

Further, the chromosomal DNA prepared in Example 39(1) was digested with

restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 181 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 182 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 982 to 1449 of the nucleotide sequence shown in SEQ ID NO: 148 was provided.

(3) Sequence analysis of the present invention DNA (A15)

The nucleotide sequence shown in SEQ ID NO: 148 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 39(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 143) consisting of 1230 nucleotides (inclusive of the stop codon) and encoding a 409 amino acid residue (SEQ ID NO: 138) and a nucleotide sequence (SEQ ID NO: 158) consisting of 207 nucleotides (inclusive of the stop codon) and encoding a 68 amino acid residue (SEQ ID NO: 153). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 138) encoded by the nucleotide sequence shown in SEQ ID NO: 143 was calculated to be 45116Da. Further, the molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 153) encoded by the nucleotide sequence shown in SEQ ID NO: 158 was calculated to be 7179Da.

Example 40 Expression of the Present Invention DNA (A15) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A15)

PCR was conducted similarly to Example 32(1), other than utilizing as a template the chromosomal DNA of Streptomyces olivochromogenes IFO 12444 prepared in Example 39(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 184 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 185. Similarly to Example 32(1), the DNA was purified from the PCR reaction solution and cloned into cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was analyzed with oligonucleotides having nucleotide sequences shown, respectively, in SEQ ID NOs: 57, 59 and 189. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 148 was designated as pCR1555AF. Similarly to Example 32(1), pCR1555AF was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 148, in which the DNA encoding the present invention protein (A15) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN1555AF"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN1555AF.

(2) Expression of the present invention protein (A15) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN1555AF and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained

from E. coli JM109/pKSN1555AF is referred to as "E. coli pKSN1555AF extract " and the supernatant fraction obtained from JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. Other than utilizing the supernatant fractions recovered in Example 40(2) (E. coli pKSN1555AF extract or E. coli pKSN2 extract), the reaction solutions were prepared similarly to Example 32(3). The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN1555AF extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 41 Metabolism of Compounds by the Present Invention Protein (A1)

(1) Preparation of plastid fractions

A hundred grams (100g) of Radish greens seeds (Takii Seed) were sawed into a dampened paper laboratory wipe in a tray, cultivated at 25℃ for 6 days in the dark and then cultivated for 4 hours under a fluorescent lamp. Thirty grams (30g) of the newly greened cotyledons were ground with a Nissei AM-8 homoginizer (Nihonseiki Seisakusho; 18,000 to 20,000rpm, 4℃, 5 seconds) in disruption buffer (1mM magnesium chloride, 20mM N-tris (hydroxymethyl)methyl-2-aminoethanesulfonate, 10mM N-2-hydroxyethylpiperidine-N'-2-ethanesulfonate, 0.5mM EDTA, 5mM cysteine, 0.5M

sucrose; pH7.7). The obtained cell lysate solution was passed through 4 layers of nylon gause. The obtained solution was centrifuged (13,170xg, 4℃, 1 minute). The obtained residue fractions were suspended with 60ml of disruption buffer and centrifuged (2,640xg, 4℃, 2 minutes). The residue fractions were resuspended in 10ml of disruption buffer, were layered with the high density buffer (1mM magnesium chloride, 20mM N-tris (hydroxymethyl)methyl-2-aminoethanesulfonate, 30mM N-2-hydroxyethylpiperidine-N'-2-ethanesulfonate, 0.5mM EDTA, 5mM cysteine, 0.6M sucrose; pH7.7) in a centrifuge tube, and were centrifuged (675xg, 4℃, 15 minutes). The residues were suspended in 3ml of suspension buffer (1mM magnesium chloride, 20mM N-tris (hydroxymethyl)methyl-2-aminoethanesulfonate, 30mM N-2-hydroxyethylpiperidine-N'-2-ethanesulfonate, 0.5mM EDTA; pH7.7) and were designated as a plastid fraction.

(2) Metabolism of compound (XII) by the present invention protein (A1)

There was prepared 100μl of a reaction solution of 50mM potassium phosphate buffer (pH7.0) containing 5ppm of compound (XII), 3mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 1mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 0.15U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 20μl of the supernatant fraction recovered in Example 4(2). The reaction solution was maintained at 30℃ for 10 minutes. Further, there was prepared and maintained similarly 100μl of a reaction solution of a 50mM potassium phosphate buffer (pH 7.0) having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B, component C and the supernatant fraction prepared in Example 4(2). Ten microliters (10μl) of 2N HCl and 500μl of ethyl acetate were added and mixed into each of the reaction solutions after the maintenance.

The resulting reaction solutions were centrifuged at 8,000xg to recover 490μl of the ethyl acetate layer. After drying the ethyl acetate layers under reduced pressure, the residue was dissolved in 100μl of 50mM of potassium phosphate buffer (pH7.0). Forty microliters (40μl) of the fraction solutions (hereinafter, the fraction solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 4(2) is referred to as "(XII) metabolism solution (A1)"; further, the fraction solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 4(2) is referred to as "(XII) control solution (A1)") were analyzed on a HPLC. Compared to the concentration of compound (XII) detected from (XII) control solution (A1), the concentration of compound (XII) detected from (XII) metabolism solution (A1) was lower. Further a peak, which was not detected from the (XII) control solution (A1), was detected from the (XII) metabolism solution (A1). Mass spectrometry was conducted for the compound contained in such a peak. The mass of the compound contained in such a peak was 14 smaller than the mass of compound (XII).

Twenty microliters (20μl) of a 32-fold dilution of the above (XII) metabolism solution (A1) and 60μl of the plastid fraction prepared in Example 41(1) were mixed. In darkened conditions, 20 μl of substrate solution (10mM adenosine triphosphate, 5mM aminolevulinic acid, 4mM glutathion reductase and 0.6mM NAD^+; pH6.5; hereinafter, such a substrate solution is referred to as "PPO substrate solution") were added and maintained at 30℃ for 1.5 hours. Further, instead of said 20μl of the 32-fold dilution of (XII) metabolism solution (A1), a reaction solution to which 20μl of the 32-fold dilution of (XII) control solution (A1) was added was prepared, and the PPO substrate solution was added and maintained similarly. Three hundred (300μl) of a dimethylsulfoxide-methanol mixture (dimethylsulfoxide: methanol = 7:3) was added to each of the reaction

solutions after the maintenance and centrifuged (8000xg, 4°C, 10 minutes). The supernatants were recovered and were subjected to reverse phase HPC analysis under the analysis conditions below to measure the amount of PPIX. The PPIX amount in the reaction solution to which (XII) metabolism solution (A1) was added was more than the PPIX amount in the reaction solution to which (XII) control solution (A1) was added.

(HPLC analysis condition 2)

column: SUMIPAX ODS212 (Sumika Chemical Analysis Service)

flow rate: 2ml/minute

detection wave length: fluorescent Ex:410nm Em:630nm

eluent: 95:5 mixture of methanol and 1M ammonium acetate (pH5.7)

(3) Metabolism of compound (XIII) by the present invention protein (A1)

Other than utilizing 5ppm of compound (XIII) instead of 5ppm of compound (XII), reaction solutions were prepared and maintained similarly to the method described in Example 41(2). Similarly to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residues were dissolved in 100μl of dimethylsulfoxide. The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 4(2) is referred to as "(XIII) metabolism solution (A1)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 4(2) is referred to as "(XIII) control solution (A1)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XIII) detected from (XIII) control solution (A1), the concentration of compound (XIII) detected from (XIII) metabolism solution (A1) was lower. Further a peak, which was not

detected from the (XIII) control solution (A1), was detected from the (XIII) metabolism solution (A1). Mass spectrometry was conducted for the compound contained in such a peak. The mass of the compound contained in such a peak was 14 smaller than the mass of compound (XIII).

Twenty microliters (20μl) of a 128-fold dilution of the above (XIII) metabolism solution (A1) and 60μl of the plastid fraction were mixed. In darkened conditions, 20 μl of PPO substrate solution were added and maintained at 30℃ for 1.5 hours. Further, instead of said 20μl of the 128-fold dilution of (XIII) metabolism solution (A1), a reaction solution to which 20μl of the 128-fold dilution of (XIII) control solution (A1) was added was prepared, and the PPO substrate solution was added and maintained similarly. Similar to Example 41(2), each of the reaction solutions after the maintenance were prepared and subjected to reverse phase HPLC analysis under the above analysis condition 2 to measure the amount of PPIX. The PPIX amount in the reaction solution to which (XIII) metabolism solution (A1) was added was more than the PPIX amount in the reaction solution to which (XIII) control solution (A1) was added.

(4) Metabolism of compound (XVI) by the present invention protein (A1)

Other than utilizing 12.5ppm of compound (XVI) instead of 5ppm of compound (XII), reaction solutions were prepared and maintained similarly to the method described in Example 41(2). Similarly to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residues were dissolved in 200μl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 4(2) is referred to as "(XVI) metabolism solution (A1)"; further, the solution derived from the reaction

solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 4(2) is referred to as "(XVI) control solution (A1)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XVI) detected from (XVI) control solution (A1), the concentration of compound (XVI) detected from (XVI) metabolism solution (A1) was lower. Further a peak, which was not detected from the (XVI) control solution (A1), was detected from the (XVI) metabolism solution (A1).

Twenty microliters (20μl) of a 8-fold dilution of the above (XVI) metabolism solution (A1) and 60μl of the plastid fraction were mixed. In darkened conditions, 20 μl of PPO substrate solution were added and maintained at 30℃ for 1.5 hours. Further, instead of said 20μl of the 8-fold dilution of (XVI) metabolism solution (A1), a reaction solution to which 20μl of the 8-fold dilution of (XVI) control solution (A1) was added was prepared, and the PPO substrate solution was added and maintained similarly. Similar to Example 41(2), each of the reaction solutions after the maintenance were prepared and subjected to reverse phase HPLC analysis under the above analysis condition 2 to measure the amount of PPIX. The PPIX amount in the reaction solution to which (XVI) metabolism solution (A1) was added was more than the PPIX amount in the reaction solution to which (XVI) control solution (A1) was added.

(5) Metabolism of compound (XVII) by the present invention protein (A1)

Other than utilizing 12.5ppm of compound (XVII) instead of 5ppm of compound (XII), reaction solutions were prepared and maintained similarly to the method described in Example 41(2). Similarly to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residues were dissolved in 200μl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter,

the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 4(2) is referred to as "(XVII) metabolism solution (A1)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 4(2) is referred to as "(XVII) control solution (A1)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XVII) detected from (XVII) control solution (A1), the concentration of compound (XVII) detected from (XVII) metabolism solution (A1) was lower. Further a peak, which was not detected from the (XVII) control solution (A1), was detected from the (XVII) metabolism solution (A1).

Twenty microliters (20μl) of a 32-fold dilution of the above (XVII) metabolism solution (A1) and 60μl of the plastid fraction were mixed. In darkened conditions, 20μl of PPO substrate solution were added and maintained at 30℃ for 1.5 hours. Further, instead of said 20μl of the 32-fold dilution of (XVII) metabolism solution (A1), a reaction solution to which 20μl of the 32-fold dilution of (XVII) control solution (A1) was added was prepared, and the PPO substrate solution was added and maintained similarly. Similar to Example 41(2), each of the reaction solutions after the maintenance were prepared and subjected to reverse phase HPLC analysis under the above analysis condition 2 to measure the amount of PPIX. The PPIX amount in the reaction solution to which (XVII) metabolism solution (A1) was added was more than the PPIX amount in the reaction solution to which (XVII) control solution (A1) was added.

(6) Metabolism of compound (VI) by the present invention protein (A1)

E. coli JM109/pKSN657F was cultured overnight at 37℃ in 3ml of TB medium containing 50μg/ml of ampicillin. A milliliter (1ml) of the obtained culture medium was

transferred to 100ml of TB medium containing 50μg/ml of ampicillin and cultured at 26°C. When OD660 reached about 0.5, 5-aminolevulinic acid was added to the final concentration of 500μM, and the culturing was continued. Thirty (30) minutes thereafter, IPTG was added to a final concentration of 1mM, and there was further culturing for 20 hours.

The cells were recovered from the culture medium, washed with 0.1M tris-HCl buffer (pH7.5) and suspended in 10ml of 0.1M Tris-HCl buffer containing 1% glucose. Compound (VI) was added to the obtained cell suspension to a final concentration of 100ppm and that was incubated with shaking at 30°C. At each of 0 hours after and 1 day after the start of shaking, 2ml of the cell suspension were fractioned. Fifty microliters (50μl) of 2N HCl were added to each and those were extracted with 2ml of ethyl acetate. The obtained ethyl acetate layers were analyzed on a HPLC under reaction condition 1. Compared to the concentration of compound (VI) detected from the ethyl acetate layer prepared from the cell suspension at 0 hours after the start of shaking, the concentration of compound (VI) detected from the ethyl acetate later prepared from the cell suspension at 1 day after the start of shaking was lower. Further a peak, which was not detected from the ethyl acetate layer prepared from the cell suspension at 0 hours after the start of shaking, was detected from the ethyl acetate layer prepared from the cell suspension at 1 day after the start of shaking. Mass spectrometry of the compound contained in said peak was conducted. The mass of the compound contained in said peak was 14 less than the mass of compound (VI).

(7) Metabolism of compound (VIII) by the present protein (A1)

Other than utilizing compound (VIII) instead of compound (VI), there was conducted in accordance with the method described in Example 41(6), a culturing of E.

coli JM109/pKSN657F, preparation of the cell suspension solution, incubation with shaking of the cell suspension solution to which compound (VIII) was added, reagent preparation from the cell suspension solution and HPLC analysis of the reagents. Compared to the concentration of compound (VIII) detected from the ethyl acetate layer prepared from the cell suspension at 0 hours after the start of shaking, the concentration of compound (VIII) detected from the ethyl acetate layer prepared from the cell suspension at 1 day after the start of shaking was lower. Further two peaks, which were not detected from the ethyl acetate layer prepared from the cell suspension at 0 hours after the start of shaking, were detected from the ethyl acetate layer prepared from the cell suspension at 1 day after the start of shaking. Mass spectrometry of the compounds contained in said peaks were conducted. The mass of the compound contained in one of said peaks was 14 less and the mass of the compound contained in the other peak was 28 less than the mass of compound (VIII).

(8) Metabolism of compound (X) by the present invention protein (A1)

Other than utilizing compound (X) instead of compound (VI), there was conducted in accordance with the method described in Example 41(6), a culturing of E. coli JM109/pKSN657F, preparation of the cell suspension solution, shake culturing of the cell suspension solution to which compound (X) was added, reagent preparation from the cell suspension solution and HPLC analysis of the reagents. Compared to the concentration of compound (X) detected from the ethyl acetate layer prepared from the cell suspension at 0 hours after the start of shaking, the concentration of compound (X) detected from the ethyl acetate later prepared from the cell suspension at 1 day after the start of shaking was lower. Further two peaks, which were not detected from the ethyl acetate layer prepared from the cell suspension at 0 hours after the start of shaking, were

detected from the ethyl acetate layer prepared from the cell suspension at 1 day after the start of shaking. Mass spectrometry of the compounds contained in said peaks was conducted. The mass of the compound contained in one of said peaks was 40 less and the mass of the compound contained in the other peak was 54 less than the mass of compound (X).

(9) Metabolism of compound (XI) by the present invention protein (A1)

Other than utilizing compound (XI) instead of compound (VI), there was conducted in accordance with the method described in Example 41(6), a culturing of E. coli JM109/pKSN657F, preparation of the cell suspension solution, shake culturing of the cell suspension solution to which compound (XI) was added, reagent preparation from the cell suspension solution and HPLC analysis of the reagents. Compared to the concentration of compound (XI) detected from the ethyl acetate layer prepared from the cell suspension at 0 hours after the start of shaking, the concentration of compound (XI) detected from the ethyl acetate layer prepared from the cell suspension at 1 day after the start of shaking was lower. Further two peaks, which were not detected from the ethyl acetate layer prepared from the cell suspension at 0 hours after the start of shaking, were detected from the ethyl acetate layer prepared from the cell suspension at 1 day after the start of shaking. Mass spectrometry of the compounds contained in said peaks was conducted. The mass of the compound contained in one of said peaks was 14 less and the mass of the compound contained in the other peak was 16 less than the mass of compound (XI).

Example 42 Metabolism of Compounds by the Present Invention Protein (A11)

(1) Metabolism of compound (X) by the present invention compound (A11)

Each of E. coli JM109/pKSN849AF and E. coli JM109/pKSN2 was cultured overnight at 37℃ in 3ml of TB culture containing 50μg/ml of ampicillin. A milliliter (1ml) of the obtained culture mediums was transferred to 100ml of TB medium containing 50μg/ml of ampicillin and cultured at 26℃. When OD660 reached about 0.5, 5-aminolevulinic acid was added to the final concentration of 500μM, and the culturing was continued. Thirty (30) minutes thereafter, IPTG was added to a final concentration of 1mM, and there was further culturing for 18 hours.

The cells were recovered from the culture medium, washed with 0.1M tris-HCl buffer (pH7.5) and suspended in 10ml of 0.1M Tris-HCl buffer containing 1% glucose. Compound (X) was added to the obtained cell suspension to a final concentration of 25ppm and that was incubated with shaking at 30℃. At each of 0 hours after and 4 days after the start of shaking, 2ml of the cell suspension were fractioned. Fifty microliters (50μl) of 2N HCl were added to each and those were extracted with 2ml of ethyl acetate. The obtained ethyl acetate layers were analyzed on a HPLC under reaction condition 1. Compared to the concentration of compound (X) detected from the ethyl acetate layer prepared from the JM109/pKSN2 cell suspension, the concentration of compound (X) detected from the ethyl acetate layer prepared from the JM109/pKSN849AF cell suspension was lower. Further 3 peaks, which were not detected from the ethyl acetate layer prepared from the JM109/pKSN2 cell suspension, were detected from the ethyl acetate layer prepared from the JM109/pKSN849AF cell suspension. Of the 3 peaks, the elution time in the HPLC of 1 of the peaks matched with the elution time of a peak of a compound that has a mass of 40 less than compound (X) detected in Example 41(8). Further, the elution time in the HPLC of another peak matched with the elution time of a peak of a compound that has a mass of 54 less than compound (X) detected in Example 41(8).

After drying, respectively, 1ml of the ethyl acetate layer prepared from the above JM109/pKSN2 cell suspension and 1ml of the ethyl acetate layer prepared from the above JM109/pKSN849AF cell suspension, the residues were dissolved in 1ml of dimethylsulfoxide (hereinafter, the solution derived from the ethyl acetate layer prepared from JM109/pKSN849AF is referred to as "(X) metabolism solution (A11)"; further, the solution derived from the ethyl acetate layer prepared from JM109/pKSN2 cell suspension is referred to as "(X) control solution (A11)").

Twenty microliters (20μl) of a 128-fold dilution of the above (X) metabolism solution (A11) and 60μl of the plastid fraction were mixed. In darkened conditions, 20μl of PPO substrate solution were added and maintained at 30℃ for 1.5 hours. Further, instead of said 20μl of the 128-fold dilution of (X) metabolism solution (A11), a reaction solution to which 20μl of the 128-fold dilution of (X) control solution (A11) was added was prepared, and the PPO substrate solution was added and maintained similarly. Similar to Example 41(2), each of the reaction solutions after the maintenance were prepared and subjected to reverse phase HPLC analysis under the above analysis condition 2 to measure the amount of PPIX. The PPIX amount in the reaction solution to which (X) metabolism solution (A11) was added was more than the PPIX amount in the reaction solution to which (X) control solution (A11) was added.

(2) Metabolism of compound (XII) by the present invention protein (A11)

Other than utilizing 20μl of the supernatant fraction recovered in Example 32(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(2). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in

100μl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 32(2) is referred to as "(XII) metabolism solution (A11)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 32(2) is referred to as "(XII) control solution (A11)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XII) detected from (XII) control solution (A11), the concentration of compound (XII) detected from (XII) metabolism solution (A11) was lower. Further a peak, which was not detected from the (XII) control solution (A11), was detected from the (XII) metabolism solution (A11). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XII) detected from (XII) metabolism solution (A1) in Example 41(2).

(3) Metabolism of compound (XIII) by the present invention protein (A11)

Other than utilizing 20μl of the supernatant fraction recovered in Example 32(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(3). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of dimethylsulfoxide. The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 32(2) is referred to as "(XIII) metabolism solution (A11)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in

Example 32(2) is referred to as "(XIII) control solution (A11)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XIII) detected from (XIII) control solution (A11), the concentration of compound (XIII) detected from (XIII) metabolism solution (A11) was lower. Further a peak, which was not detected from the (XIII) control solution (A11), was detected from the (XIII) metabolism solution (A11). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XIII) detected from (XIII) metabolism solution (A11) in Example 41(3).

(4) Metabolism of compound (XVI) by the present invention protein (A11)

Other than utilizing 20μl of the supernatant fraction recovered in Example 32(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(4). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 200μl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 32(2) is referred to as "(XVI) metabolism solution (A11)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 32(2) is referred to as "(XVI) control solution (A11)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XVI) detected from (XVI) control solution (A11), the concentration of compound (XVI) detected from (XVI) metabolism solution (A11) was lower. Further a peak, which was not detected from the (XVI) control solution (A11),

was detected from the (XVI) metabolism solution (A11). The elution time of said peak on the HPLC matched an elution time of a peak in Example 41(4) which was detected from (XVI) metabolism solution (A11) and not detected in (XVI) control solution (A11).

(5) Metabolism of compound (XVII) by the present invention protein (A11)

Other than utilizing 20µl of the supernatant fraction recovered in Example 32(2) instead of 20µl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(5). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 200µl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20µl of supernatant fraction recovered in Example 32(2) is referred to as "(XVII) metabolism solution (A11)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 32(2) is referred to as "(XVII) control solution (A11)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XVII) detected from (XVII) control solution (A11), the concentration of compound (XVII) detected from (XVII) metabolism solution (A11) was lower. Further a peak, which was not detected from the (XVII) control solution (A11), was detected from the (XVII) metabolism solution (A11). The elution time of said peak on the HPLC matched an elution time of a peak in Example 41(5) which was detected from (XVII) metabolism solution (A1) and not detected in (XVII) control solution (A1).

Example 43 Metabolism of compounds by the present invention protein (A2), (A3),

(A12), (A13), (A14) or (A15) or the present protein (A10)

(1) Metabolism of compound (XII) by the present invention protein (A2)

Other than utilizing 20µl of the supernatant fraction recovered in Example 7(2) instead of 20µl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(2). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100µl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20µl of supernatant fraction recovered in Example 7(2) is referred to as "(XII) metabolism solution (A2)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 7(2) is referred to as "(XII) control solution (A2)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XII) detected from (XII) control solution (A2), the concentration of compound (XII) detected from (XII) metabolism solution (A2) was lower. Further a peak, which was not detected from the (XII) control solution (A2), was detected from the (XII) metabolism solution (A2). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XII) detected from (XII) metabolism solution (A1) in Example 41(2).

(2) Metabolism of compound (XII) by the present invention protein (A3)

Other than utilizing 20µl of the supernatant fraction recovered in Example 12(2) instead of 20µl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in

Example 41(2). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 12(2) is referred to as "(XII) metabolism solution (A3)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 7(2) is referred to as "(XII) control solution (A3)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XII) detected from (XII) control solution (A3), the concentration of compound (XII) detected from (XII) metabolism solution (A3) was lower. Further a peak, which was not detected from the (XII) control solution (A3), was detected from the (XII) metabolism solution (A3). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XII) detected from (XII) metabolism solution (A1) in Example 41(2).

(3) Metabolism of compound (XII) by the present protein (A10)

Other than utilizing 20μl of the supernatant fraction recovered in Example 10(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(2). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 10(2) is referred to

as "(XII) metabolism solution (A10)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 12(3) is referred to as "(XII) control solution (A10)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XII) detected from (XII) control solution (A10), the concentration of compound (XII) detected from (XII) metabolism solution (A10) was lower. Further a peak, which was not detected from the (XII) control solution (A10), was detected from the (XII) metabolism solution (A10). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XII) detected from (XII) metabolism solution (A1) in Example 41(2).

(4) Metabolism of compound (XII) by the present invention protein (A12)

Other than utilizing 20μl of the supernatant fraction recovered in Example 34(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(2). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 34(2) is referred to as "(XII) metabolism solution (A12)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 34(2) is referred to as "(XII) control solution (A12)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XII) detected from (XII) control solution (A12), the

concentration of compound (XII) detected from (XII) metabolism solution (A12) was lower. Further a peak, which was not detected from the (XII) control solution (A12), was detected from the (XII) metabolism solution (A12). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XII) detected from (XII) metabolism solution (A1) in Example 41(2).

(5) **Metabolism of compound (XII) by the present invention protein (A13)**

Other than utilizing 20μl of the supernatant fraction recovered in Example 36(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(2). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 36(2) is referred to as "(XII) metabolism solution (A13)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 36(2) is referred to as "(XII) control solution (A13)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XII) detected from (XII) control solution (A13), the concentration of compound (XII) detected from (XII) metabolism solution (A13) was lower. Further a peak, which was not detected from the (XII) control solution (A13), was detected from the (XII) metabolism solution (A13). The elution time of the said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XII) detected from (XII) metabolism solution (A1) in Example

41(2).

(6) **Metabolism of compound (XII) by the present invention protein (A14)**

Other than utilizing 20μl of the supernatant fraction recovered in Example 38(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(2). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 38(2) is referred to as "(XII) metabolism solution (A14)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 38(2) is referred to as "(XII) control solution (A14)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XII) detected from (XII) control solution (A14), the concentration of compound (XII) detected from (XII) metabolism solution (A14) was lower. Further a peak, which was not detected from the (XII) control solution (A14), was detected from the (XII) metabolism solution (A14). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XII) detected from (XII) metabolism solution (A1) in Example 41(2).

(7) **Metabolism of compound (XII) by the present invention protein (A15)**

Other than utilizing 20μl of the supernatant fraction recovered in Example 40(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction

solutions were prepared and maintained in accordance with the method described in Example 41(2). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of 50mM potassium phosphate buffer (pH7.0). The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 40(2) is referred to as "(XII) metabolism solution (A15)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 40(2) is referred to as "(XII) control solution (A15)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XII) detected from (XII) control solution (A15), the concentration of compound (XII) detected from (XII) metabolism solution (A15) was lower. Further a peak, which was not detected from the (XII) control solution (A15), was detected from the (XII) metabolism solution (A15). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XII) detected from (XII) metabolism solution (A1) in Example 41(2).

(8) Metabolism of compound (XIII) by the present invention protein (A2)

Other than utilizing 20μl of the supernatant fraction recovered in Example 7(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(3). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of dimethylsulfoxide. The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl

of supernatant fraction recovered in Example 7(2) is referred to as "(XIII) metabolism solution (A2)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 7(2) is referred to as "(XIII) control solution (A2)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XIII) detected from (XIII) control solution (A2), the concentration of compound (XIII) detected from (XIII) metabolism solution (A2) was lower. Further a peak, which was not detected from the (XIII) control solution (A2), was detected from the (XIII) metabolism solution (A2). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XIII) detected from (XIII) metabolism solution (A1) in Example 41(3).

(9) Metabolism of compound (XIII) by the present invention protein (A3)

Other than utilizing 20μl of the supernatant fraction recovered in Example 12(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(3). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of dimethylsulfoxide. The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 12(2) is referred to as "(XIII) metabolism solution (A3)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 12(2) is referred to as "(XIII) control solution (A3)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XIII)

detected from (XIII) control solution (A3), the concentration of compound (XIII) detected from (XIII) metabolism solution (A3) was lower. Further a peak, which was not detected from the (XIII) control solution (A3), was detected from the (XIII) metabolism solution (A3). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XIII) detected from (XIII) metabolism solution (A1) in Example 41(3).

(10) Metabolism of compound (XIII) by the present protein (A10)

Other than utilizing 20µl of the supernatant fraction recovered in Example 10(2) instead of 20µl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(3). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100µl of dimethylsulfoxide. The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20µl of supernatant fraction recovered in Example 10(2) is referred to as "(XIII) metabolism solution (A10)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 10(2) is referred to as "(XIII) control solution (A10)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XIII) detected from (XIII) control solution (A10), the concentration of compound (XIII) detected from (XIII) metabolism solution (A10) was lower. Further a peak, which was not detected from the (XIII) control solution (A10), was detected from the (XIII) metabolism solution (A10). The elution time of the said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said

compound (XIII) detected from (XIII) metabolism solution (A1) in Example 41(3).

(11) Metabolism of compound (XIII) by the present invention protein (A12)

Other than utilizing 20μl of the supernatant fraction recovered in Example 34(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(3). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of dimethylsulfoxide. The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 34(2) is referred to as "(XIII) metabolism solution (A12)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 34(2) is referred to as "(XIII) control solution (A12)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XIII) detected from (XIII) control solution (A12), the concentration of compound (XIII) detected from (XIII) metabolism solution (A12) was lower. Further a peak, which was not detected from the (XIII) control solution (A12), was detected from the (XIII) metabolism solution (A12). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XIII) detected from (XIII) metabolism solution (A1) in Example 41(3).

(12) Metabolism of compound (XIII) by the present invention protein (A13)

Other than utilizing 20μl of the supernatant fraction recovered in Example 36(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction

solutions were prepared and maintained in accordance with the method described in Example 41(3). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of dimethylsulfoxide. The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 36(2) is referred to as "(XIII) metabolism solution (A13)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 36(2) is referred to as "(XIII) control solution (A13)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XIII) detected from (XIII) control solution (A13), the concentration of compound (XIII) detected from (XIII) metabolism solution (A13) was lower. Further a peak, which was not detected from the (XIII) control solution (A13), was detected from the (XIII) metabolism solution (A13). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XIII) detected from (XIII) metabolism solution (A1) in Example 41(3).

(13) Metabolism of compound (XIII) by the present invention protein (A14)

Other than utilizing 20μl of the supernatant fraction recovered in Example 38(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solution were prepared and maintained in accordance with the method described in Example 41(3). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of dimethylsulfoxide. The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl

of supernatant fraction recovered in Example 38(2) is referred to as "(XIII) metabolism solution (A14)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 38(2) is referred to as "(XIII) control solution (A14)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of compound (XIII) detected from (XIII) control solution (A14), the concentration of compound (XIII) detected from (XIII) metabolism solution (A14) was lower. Further a peak, which was not detected from the (XIII) control solution (A14), was detected from the (XIII) metabolism solution (A14). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XIII) detected from (XIII) metabolism solution (A1) in Example 41(3).

(14) Metabolism of compound (XIII) by the present invention protein (A15)

Other than utilizing 20μl of the supernatant fraction recovered in Example 40(2) instead of 20μl of the supernatant fraction recovered in Example 4(2), the reaction solutions were prepared and maintained in accordance with the method described in Example 41(3). Similar to Example 41(2), each of the reaction solutions after the maintenance was extracted with ethyl acetate and the obtained residue was dissolved in 100μl of dimethylsulfoxide. The obtained solutions (hereinafter, the solution derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 40(2) is referred to as "(XIII) metabolism solution (A15)"; further, the solution derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 40(2) is referred to as "(XIII) control solution (A15)") were analyzed on a HPLC under the above analysis condition 1. Compared to the concentration of

compound (XIII) detected from (XIII) control solution (A15), the concentration of compound (XIII) detected from (XIII) metabolism solution (A15) was lower. Further a peak, which was not detected from the (XIII) control solution (A15), was detected from the (XIII) metabolism solution (A15). The elution time of said peak on the HPLC matched an elution time of a peak of a compound in which the mass is 14 less than said compound (XIII) detected from (XIII) metabolism solution (A1) in Example 41(3).

Example 44 Preparation of the Present Invention Antibody (A) Recognizing the Present Invention Protein (A1) (hereinafter referred to as "present invention antibody (A1)")

(1) Preparation of the extract of an E. coli expressing the present invention protein (A1)

In accordance with the method described in Example 4(2), E. coli JM109/pKSN657F, which expresses the present invention protein (A1), was pre-cultured overnight and then cultured in IL of TB medium containing 50μg/ml of ampicillin. After recovering and disrupting the cells, supernatant fractions (E. coli pKSN657F extract) were prepared from the obtained cell lysate solution.

(2) Purification of the present invention protein (A1)

The present invention protein (A1) was purified according to the method described in Example 2(4) by subjecting the supernatant fraction obtained in Example 44(1) (E. coli pKSN657F extract) in turn to a Hiload HiLoad26/10 Q Sepharose HP column and then a Bio-Scale Ceramic Hydroxyapatite, Type I column CHT10-1 column. The purified fractions were analyzed on a 10% to 20% SDS-PAGE, to confirm that those were fractions of only the present invention protein (A1).

(3) **Preparation of the present invention antibody (A1)**

The present invention protein (A1) prepared in Example 44(2) was dissolved in 0.05M potassium phosphate buffer (pH7.0) so that the concentration was 1mg/ml. Forty microliters (40μl) of RAS (MPL (Monophosphoryl lipid A) + TDM (Synthetic Trehalose Dicorynomycolate) + CWS (Cell Wall Skeleton) Adjuvant System (Sigma Company)) already incubated at 42℃ to 43℃ was added and well mixed into 2ml of the obtained solution. The obtained mixture was administered, respectively, to New Zealand White rabbits (female, 14 weeks old, average of 2.4kg) at 1ml per rabbit. As such, 100μl was injected subcutaneously at 10 locations on the back. About 1/2 of the amount of the first administration was administered after each of 3 weeks and 5 weeks. During such time, the antibody titer was measured by sampling the blood from a ear vein of the rabbit. Since the antibody titer increased after the third administration, the immunized rabbit at 2 weeks after the third administration was exsanguinated from the neck. The obtained blood was added into a Separapit Tube (Sekisui Chemical Company), incubated at 37℃ for 2 hours and was then centrifuged (3000rpm, 20 minutes, room temperature). The antiserum (containing the present invention antibody (A1)) was obtained by recovering the supernatant.

Example 45 Detection of the Present Protein by the Present Invention Antibody (A1) and Detection of a Cell Expressing the Present Protein

An immunoblot was conducted by utilizing the present invention antibody (A1) obtained in Example 44 with each of the E. coli extracts. There was a SDS polyacrylamide electrophoresis (40mA, 1 hour) of: the E. coli pKSN657F extract obtained in Example 4(2) (containing about 0.5pmol of the present invention protein (A1),

containing about 0.78mg of protein); the E. coli pKSN2 extract obtained in Example 4(2) (containing about 0.78mg of protein) the E. coli pKSN923F extract obtained in Example 7(2) (containing about 2pmol of the present invention protein (A2)); the E. coli pKSN671F extract obtained in Example 12(2) (containing about 2pmol of the present invention protein (A3)); the E. coli pKSN646F extract obtained in Example 27(2) (containing about 2pmol of the present invention protein (A4)); the E. coli pKSN11796F extract obtained in Example 10(2) (containing about 2pmol of the present protein (A10)); the E. coli pKSNSCA extract obtained in Example 14(2) (containing about 2pmol of the present protein (A9)); the E. coli pKSN849AF extract obtained in Example 32(2) (containing about 2pmol of the present invention protein (A11)); the E. coli pKSN1618F extract obtained in Example 34(2) (containing about 2pmol of the present invention protein (A12)); the E. coli pKSN474F extract obtained in Example 36(2) (containing about 2pmol of the present invention protein (A13)); the E. coli pKSN1491AF extract obtained in Example 38(2) (containing about 2pmol of the present invention protein (A14)); and the E. coli pKSN1555AF extract obtained in Example 40(2) (containing about 2pmol of the present invention protein (A15)). A PVDF membrane was placed on the gel. The proteins in the gel were transferred onto the PVDF membrane by a treatment with a BioRad blotting device at 4℃, 30V for 2 hours, while in the condition of being soaked in transfer buffer (25mM Tris, 192mM glycine, 10% methanol). After washing with TBS + Tween 20 solution (50mM Tris-HCl (pH7.5), 200mM NaCl, 0.05% Tween 20), the obtained PVDF membrane was incubated for 30 minutes in TBS + Tween 20 solution containing 3% BSA and was then utilized for a reaction with the above antiserum diluted 30,000 fold for 30 minutes in TBS + Tween 20 solution containing 3% BSA. After the reaction, the PVDF membrane was washed twice with TBS + Tween 20 solution. The PVDF membrane was then utilized for a reaction in TBS + Tween 20

solution containing 3% BSA for 30 minutes with a 3000 fold dilution of anti-rabbit IgG goat anti-serum labeled with alkaline phosphatase (Santa Cruz Biotechnology Company). After the reaction, the PVDF membrane was washed twice with TBS + Tween 20 solution and was soaked in NBT-BCIP solution (Sigma Company). There was detected a stain for a band corresponding to each of the present invention proteins (A1), (A2), (A3), (A4), (A11), (A12), (A13), (A14) and (A15) as well as the present proteins (A9) and (A10). No stained band was detected with the reagent of E. coli pKSN2 extract (containing about 0.78mg of protein) obtained in Example 4(2).

Example 46 Preparation and Expression of the Present Invention DNA (A1) inwhich the Codon usage has been Adjusted for Expression in Soybean (hereinafter referred to as the "present invention DNA (A1)S")

(1) Preparation of the present invention DNA (A1)S

PCR was conducted with Pyrobest DNA polymerase (Takara Shuzo Company) according to the attached manual, by utilizing a primer having a nucleotide sequence shown in SEQ ID NO: 192 and a primer having a nucleotide sequence shown in SEQ ID NO: 213. An aliquot of the obtained PCR product was utilized as a template for a PCR conducted similarly utilizing a primer having the nucleotide sequence shown in SEQ ID NO: 191 and a primer having the nucleotide sequence shown in SEQ ID NO: 212. Further, an aliquot of that PCR product was utilized as a template for a PCR conducted similarly utilizing a primer having the nucleotide sequence shown in SEQ ID NO: 190 and a primer having the nucleotide sequence shown in SEQ ID NO: 211. The obtained reaction solution was designated as reaction solution 1.

PCR was conducted with Pyrobest DNA polymerase (Takara Shuzo Company) according to the attached manual, by utilizing a primer having a nucleotide sequence

shown in SEQ ID NO: 195 and a primer having a nucleotide sequence shown in SEQ ID NO: 210. An aliquot of the obtained PCR product was utilized as a template for a PCR conducted similarly utilizing a primer having the nucleotide sequence shown in SEQ ID NO: 194 and a primer having the nucleotide sequence shown in SEQ ID NO: 209. Further, an aliquot of that PCR product was utilized as a template for a PCR conducted similarly utilizing a primer having the nucleotide sequence shown in SEQ ID NO: 193 and a primer having the nucleotide sequence shown in SEQ ID NO: 208. The obtained reaction solution was designated as reaction solution 2.

PCR was conducted with Pyrobest DNA polymerase (Takara Shuzo Company) according to the attached manual by utilizing a primer having a nucleotide sequence shown in SEQ ID NO: 198 and a primer having a nucleotide sequence shown in SEQ ID NO: 207. An aliquot of the obtained PCR product was utilized as a template for a PCR conducted similarly utilizing a primer having the nucleotide sequence shown in SEQ ID NO: 197 and a primer having the nucleotide sequence shown in SEQ ID NO: 206. Further, an aliquot of that PCR product was utilized as a template for a PCR conducted similarly utilizing a primer having the nucleotide sequence shown in SEQ ID NO: 196 and a primer having the nucleotide sequence shown in SEQ ID NO: 205. The obtained reaction solution was designated as reaction solution 3.

PCR was conducted with Pyrobest DNA polymerase (Takara Shuzo Company) according to the attached manual, by utilizing a primer having a nucleotide sequence shown in SEQ ID NO: 201 and a primer having a nucleotide sequence shown in SEQ ID NO: 204. An aliquot of the obtained PCR product was utilized as a template for a PCR conducted similarly utilizing a primer having the nucleotide sequence shown in SEQ ID NO: 200 and a primer having the nucleotide sequence shown in SEQ ID NO: 203. Further, an aliquot of that PCR product was utilized as a template for a PCR conducted

similarly utilizing a primer having the nucleotide sequence shown in SEQ ID NO: 199 and a primer having the nucleotide sequence shown in SEQ ID NO: 202. The obtained reaction solution was designated as reaction solution 4.

The reaction solutions 1 to 4 obtained in such a way were mixed. PCR was conducted with Pyrobest DNA polymerase (Takara Shuzo Company) according to the attached manual, by utilizing as a template an aliquot of the mixture thereof and by utilizing a primer having a nucleotide sequence shown in SEQ ID NO: 190 and a primer having a nucleotide sequence shown in SEQ ID NO: 202. The nucleotide sequence of the amplified DNA was confirmed. There was obtained a DNA having a sequence in which the nucleotide sequence 5'-cat-3' is connected upstream of the 5' terminus and the nucleotide sequence 5'-aagctt-3' is connected downstream of the 3' terminus of the nucleotide sequence shown in SEQ ID NO: 214.

The codon usage of the present invention DNA (A1) having the nucleotide sequence shown in SEQ ID NO: 6 (GC content of 70.58%) is shown in Table 22 and Table 23. The codon usage of soybean (GC content of 46.12%, Codon Usage Database publised by Kazusa DNA Research Institute (http//:www.kazusa.or.jp/codon)) is shown in Table 24 and Table 25. The codon usage of the present invention DNA (A1) having the nucleotide sequence shown in SEQ ID NO: 214 (GC content of 51.59%) is shown in Table 26 and Table 27.

Table 22

codon	%	codon	%
TTT	0.00	TCT	0.00
TTC	3.18	TCC	1.71
TTA	0.00	TCA	0.00
TTG	1.22	TCG	2.20
CTT	0.00	CCT	0.00
CTC	3.67	CCC	4.16
CTA	0.00	CCA	0.00
CTG	7.09	CCG	2.69
ATT	0.24	ACT	0.24
ATC	4.16	ACC	2.69
ATA	0.00	ACA	0.24
ATG	2.69	ACG	1.96
GTT	0.24	GCT	0.00
GTC	3.67	GCC	7.58
GTA	0.00	GCA	0.49
GTG	3.18	GCG	3.42

Table 23

codon	%	codon	%
TAT	0.00	TGT	0.24
TAC	1.47	TGC	0.98
TAA	0.00	TGA	0.00
TAG	0.24	TGG	0.98
CAT	0.24	CGT	1.22
CAC	2.20	CGC	4.40
CAA	0.24	CGA	0.24
CAG	2.93	CGG	4.16
AAT	0.00	AGT	0.00
AAC	1.22	AGC	0.49
AAA	0.24	AGA	0.00
AAG	0.98	AGG	0.00
GAT	0.98	GGT	0.98
GAC	7.82	GGC	3.42
GAA	0.73	GGA	0.24
GAG	5.38	GGG	1.22

Table 24

codon	%	codon	%
TTT	2.03	TCT	1.71
TTC	2.09	TCC	1.21
TTA	0.82	TCA	1.45
TTG	2.21	TCG	0.44
CTT	2.36	CCT	2.00
CTC	1.66	CCC	1.01
CTA	0.82	CCA	2.05
CTG	1.22	CCG	0.40
ATT	2.61	ACT	1.78
ATC	1.64	ACC	1.49
ATA	1.27	ACA	1.51
ATG	2.27	ACG	0.41
GTT	2.67	GCT	2.81
GTC	1.24	GCC	1.69
GTA	0.73	GCA	2.27
GTG	2.20	GCG	0.59

Table 25

codon	%	codon	%
TAT	1.61	TGT	0.72
TAC	1.53	TGC	0.75
TAA	0.11	TGA	0.09
TAG	0.06	TGG	1.21
CAT	1.33	CGT	0.72
CAC	1.09	CGC	0.63
CAA	2.04	CGA	0.38
CAG	1.71	CGG	0.27
AAT	2.10	AGT	1.21
AAC	2.27	AGC	1.08
AAA	2.63	AGA	1.42
AAG	3.83	AGG	1.35
GAT	3.29	GGT	2.17
GAC	2.06	GGC	1.38
GAA	3.35	GGA	2.23
GAG	3.46	GGG	1.29

Table 26

codon	%	codon	%
TTT	1.71	TCT	0.98
TTC	1.47	TCC	0.73
TTA	0.98	TCA	0.98
TTG	2.93	TCG	0.24
CTT	3.18	CCT	2.44
CTC	2.20	CCC	1.22
CTA	0.98	CCA	2.69
CTG	1.71	CCG	0.49
ATT	2.20	ACT	1.71
ATC	1.22	ACC	1.47
ATA	0.98	ACA	1.47
ATG	2.69	ACG	0.49
GTT	2.93	GCT	4.16
GTC	1.22	GCC	2.69
GTA	0.73	GCA	3.67
GTG	2.20	GCG	0.98

Table 27

codon	%	codon	%
TAT	0.73	TGT	0.73
TAC	0.73	TGC	0.49
TAA	0.00	TGA	0.00
TAG	0.24	TGG	0.98
CAT	1.47	CGT	1.47
CAC	0.98	CGC	1.47
CAA	1.71	CGA	0.73
CAG	1.47	CGG	0.49
AAT	0.73	AGT	0.73
AAC	0.49	AGC	0.73
AAA	0.49	AGA	2.93
AAG	0.73	AGG	2.93
GAT	5.38	GGT	1.71
GAC	3.42	GGC	1.22
GAA	2.69	GGA	1.96
GAG	3.42	GGG	0.98

(2) Production of a transformed E. coli having the present invention protein (A1)S

The DNA having the nucleotide sequence shown in SEQ ID NO: 214 obtained in Example 46(1) was digested with restriction enzymes NdeI and HindIII. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid in which the DNA having the nucleotide sequence shown in SEQ ID NO: 214 is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN657 soy"). Said plasmid was introduced into E. coli JM109. The obtained E. coli transformant was designated JM109/pKSN657soy.

(3) Expression of the present invention protein (A1) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN657soy obtained in Example 46(2) and E. coli JM109/pKSN657 obtained in Example 4(1) was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN657soy is referred to as "E. coli pKSN849soy extract " and the supernatant fraction obtained from E. coli JM109/pKSN657 is referred to as "E. coli pKSN657 extract "). The amount of P450 per the protein amount contained in E. coli pKSN657soy extract was compared to and was higher than the amount of P450 per the protein amount contained in E. coli pKSN657 extract.

Example 47 Introduction of the Present Invention DNA (A1)S into a Plant

(1) Construction of a Chloroplast Expression Plasmid Containing the Present

Invention DNA (A1)S for Direct Introduction - part 1

A plasmid containing a chimeric DNA in which the present invention DNA (A1)S was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit without a change of frames in the codons was constructed as a plasmid for introducing the present invention DNA (A1)S into a plant with the particle gun method.

First, DNA comprising the nucleotide sequence shown in SEQ ID NO: 214 was amplified by PCR. The PCR was conducted by utilizing as a template pKSN657soy obtained in Example 46(2) and by utilizing as primers an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 394 and an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 395. The PCR utilized KOD-plus (Toyobo Company). The PCR carried out after conducting a maintenance at 94℃ for 2 minutes; 30 cycles of a cycle that included maintaining 94℃ for 30 seconds, followed by 50℃ for 30 seconds, and followed by 68℃ for 60 seconds; and a final maintenance at 68℃ for 30 seconds. The amplified DNA was recovered and purified with MagExtractor-PCR & Gel-Clean up (Toyobo Company) by conducting the procedures according to the attached manual. After digesting the purified DNA with restriction enzymes EcoT22I and SacI, the DNA comprising the nucleotide sequence shown in SEQ ID NO: 214 was recovered. After digesting plasmid pUCrSt657 obtained in Example 16(2) with restriction enzymes EcoT22I and SacI, there was isolated a DNA of about 2.9kbp having a nucleotide sequence derived from pUC19 and a sequence encoding a chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit. The obtained DNA and the above DNA comprising the nucleotide sequence shown in SEQ ID NO: 214 were ligated to obtain pUCrSt657soy (Fig. 48) containing a chimeric DNA in which the present invention DNA (A1)S was connected immediately after the nucleotide sequence

encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit without a change of frames in the codons.

The obtained plasmid pUCrSt657soy was digested with restriction enzymes BamHI and SacI to isolate a DNA comprising a nucleotide sequence shown in SEQ ID NO: 214. Said DNA was inserted between the restriction enzyme site of BglII and the restriction enzyme site of SacI of plasmid pNdG6-ΔT obtained in Example 16(2) to obtain plasmid pSUM-NdG6-rSt-657soy (Fig. 49) wherein the CR16G6 promoter has connected downstream the chimeric DNA in which the present invention DNA (A1)S was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit without a change of frames in the codons.

Next, the plasmid was introduced into E. coli DH5 α competent cells (Takara Shuzo Company) and the ampicillin resistant cells were selected. Further, the nucleotide sequences of the plasmids contained in the selected ampicillin resistant strains were determined by utilizing BigDye Terminator Cycle Sequencing Ready Reaction kit v3.0 (PE Applied Biosystems Company) and DNA sequencer 3100 (PE Applied Biosytems Company). As a result, it was confirmed that plasmid pSUM-NdG6-rSt-657soy had the nucleotide sequence shown in SEQ ID NO: 214.

(2) Construction of a chloroplast expression plasmid having the present invention DNA (A1)S for direct introduction - part (2)

A plasmid was constructed for introducing the present invention DNA (A1)S into a plant with the particle gun method. The plasmid contained a chimeric DNA in which the present invention DNA (A1)S was connected immediately after the nucleotide sequences encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small

subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frames in the codons. First, DNA comprising the nucleotide sequence shown in SEQ ID NO: 214 was amplified by PCR. The PCR was conducted by utilizing as a template pKSN657soy obtained in Example 46(2) and by utilizing as primers an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 395 and an oligonucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 396. The PCR utilized KOD-plus (Toyobo Company). The PCR carried out after conducting a maintenance at 94℃ for 2 minutes; 25 cycles of a cycle that included maintaining 94℃ for 30 seconds, followed by 46℃ for 30 seconds, and followed by 68℃ for 60 seconds; and a final maintenance at 68℃ for 3 minutes. The amplified DNA was recovered and purified with MagExtractor-PCR & Gel-Clean up (Toyobo Company) by conducting the procedures according to the attached manual. After digesting the purified DNA with restriction enzyme SacI, the DNA comprising the nucleotide sequence shown in SEQ ID NO: 214 was recovered.

Plasmid pKFrSt12-657 obtained in Example 16(3) was digested with restriction enzyme BspHI. The DNA was then blunt ended and the 5' terminus was dephosphorylated by utilizing TaKaRa BKLKit (Takara Shuzo Company) in accordance with the attached manual. Next, after the DNA was digested with restriction enzyme SacI, the DNA derived from plasmid pKFrSt12 was isolated. Said DNA was ligated with the DNA which was digested with SacI and which comprises the nucleotide sequence shown in SEQ ID NO: 214, in order to obtain plasmid pKFrSt12-657soy (Fig. 50) containing the chimeric DNA in which the present invention DNA (A1)S was connected immediately after the nucleotide sequences encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frames in the codons.

The obtained plasmid pKFrSt12-657soy was digested with restriction enzymes BamHI and SacI to isolate DNA comprising the nucleotide sequence shown in SEQ ID NO: 214. Said DNA was inserted between the restriction enzyme site of BglII and the restriction enzyme site of SacI of plasmid pNdG6-ΔT to obtain plasmid pSUM-NdG6-rSt12-657soy (Fig. 51) wherein the CR16G6 promoter has connected downstream the chimeric DNA in which said DNA was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit without a change of frames in the codons.

Next, the plasmid was introduced into E. coli DH5α competent cells (Takara Shuzo Company) and the ampicillin resistant cells were selected. Further, the nucleotide sequences of the plasmids contained in the ampicillin resistant strains were determined by utilizing BigDye Terminator Cycle Sequencing Ready Reaction kit v3.0 (PE Applied Biosystems Company) and DNA sequencer 3100 (PE Applied Biosytems Company). As a result, it was confirmed that plasmid pSUM-NdG6-rSt12-657soy had the nucleotide sequence shown in SEQ ID NO: 214.

(3) Introduction of the present invention DNA (A1)S into soybean

The globular embryos of soybeans (cultivar: Fayette and Jack) were prepared according to the method described in Example 17(1), other than substituting the vitamin source of MS medium with the vitamin source of B5 medium (O. L. Gamborg et al., Exp. Cell Res. (1986) 50 p151).

The obtained globular embryo was transplanted into fresh somatic embryo growth medium and cultured for 2 to 3 days. In accordance with the method described in Example 17(2), plasmid pSUM-NdG6-rSt-657soy constructed in Example 47(1) or plasmid pSUM-NdG6-rSt12-657soy constructed in Example 47(2) was introduced to said

globular embryos.

(4) **Selection of somatic embryo with hygromycin**

Selection by hygromycin of a globular embryo after the gene introduction obtained in Example 47(3) was conducted according to the method described in Example 17(3), other than substituting the vitamin source of MS medium with the vitamin source of B5 medium. However, after the second transplant, a medium to which 0.2(w/v)% of Gelrite was added or a liquid medium to which no Gelrite was added was utilized as the somatic embryo selection medium. In the case of the liquid medium, the culturing had 90gentle revolutions per minute.

(5) **Selection of somatic embryo with compound (II)**

Selection by compound (II) of a globular embryo after the gene introduction obtained in Example 47(3) is conducted according to the method described in Example 17(4), other than substituting the vitamin source of MS medium with the vitamin source of B5 medium.

(6) **Plant regeneration from the somatic embryo, acclimation and cultivation**

In accordance with the method described in Example 17(5), the plant regeneration is conducted from the globular embryos selected in Example 47(4) or 47(5). However, the agar concentration in the development medium is adjusted to 0.8(w/v)% or 1.0(w/v)%. Further, the vitamin source of the MS medium of the germination medium is substituted with the vitamin source of B5 medium.

The plant with roots and developed leaves undergo the acclimation and cultivation accordingly with the method described in Example 17(6) and are harvested.

(7) **Evaluation of the resistance to herbicidal compound (II)**

The degree of resistance against compound (II) of the regenerated plant obtained in Example 47(6) is evaluated in accordance with the method described in Example 17(4).

(8) **Construction of a chloroplast expression plasmid having the present invention DNA (A1)S for agrobacterium introduction**

A plasmid for introducing the present invention DNA (A1)S into a plant with the agrobacterium method is constructed. Plasmid pSUM-NdG6-rSt-657soy was digested with restriction enzyme NotI, to obtain a chimeric DNA in which the present invention DNA (A1)S was connected immediately after the nucleotide sequence encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit without a change of frames in the codons. Said DNA was inserted into the NotI restriction site of the above binary plasmid vector pBI121S obtained in Example 18 to obtain plasmid pBI-NdG6-rSt-657soy (Fig. 52). Further, plasmid pSUM-NdG6-rSt12-657soy was digested with restriction enzyme NotI, to isolate a chimeric DNA in which the present invention DNA (A1)S was connected immediately after the nucleotide sequences encoding the chloroplast transit peptide of soybean (cv. Jack) RuBPC small subunit and encoding thereafter 12 amino acids of the mature protein, without a change of frames in the codons. Such a DNA was inserted into the NotI restriction site of the above binary plasmid vector pBI121S to obtain plasmid pBI-NdG6-rSt12-657soy (Fig. 53).

(9) **Introduction of the present invention DNA (A1)S to tobacco**

The present invention DNA (A1)S was introduced into tobacco with the agrobacterium method, utilizing plasmid pBI-NdG6-rSt-657soy and pBI-NdG6-rSt12-

657soy obtained in Example 47(8).

First, in accordance with the method described in Example 19, each of the plasmids pBI-NdG6-rSt-657soy and pBI-NdG6-rSt12-657soy was introduced into Agrobacterium tumefaciens LBA4404 (Clontech Company). The transgenic agrobacterium bearing pBI-NdG6-rSt-657soy or pBI-NdG6-rSt12-657soy were isolated.

Next, other than culturing overnight the transgenic agrobacterium bearing the above plasmid at 30℃ in LB liquid medium containing 25mg/L kanamycin, said agrobacterium were utilized to introduce genes into tobacco according to the method described in Example 19. There were obtained, respectively, transgenic tobaccos which have incorporated the T-DNA region of pBI-NdG6-rSt-657soy or pBI-NdG6-rSt12-657soy.

(10) Evaluation of the resistance utilizing a leaf piece of the present invention DNA (A1)S transgenic tobacco

Leaves were taken from 35 transgenic tobaccos obtained in Example 47(9). Each leaf was divided into pieces in which each piece was 5 to 7mm wide. Leaf pieces were planted onto MS agar medium containing 0, 0.05, 0.1 or 0.2mg/L of compound (II) and cultured in the light at room temperature. On the 11th day of culturing, the herbicidal damage of each of the leaf pieces was observed. Further, leaf pieces were planted onto MS agar mediums containing 0, 0.01, 0.02, 0.05 or 0.1mg/L of compound (XII) and cultured in the light at room temperature. On the 7th day of culturing, the herbicidal damage of each of the leaf pieces was observed. As a control, 20 leaf pieces of tobacco to which no genetic introduction has been conducted (hereinafter, referred to as "wild type tobacco") were utilized on each concentration. An average score for each group was determined by scoring 1 point to a leaf piece that continuously grew, 0.5 points to a halfly

withered leaf piece in which chemical damage was observed, and 0 points to a leaf piece which turned white and had withered. The leaf pieces of the tobacco to which the present invention DNA (A1)S (the T-DNA region of plasmid pBI-NdG6-rSt-657soy or pBI-NdG6-rSt12-657soy) has been introduced provided a higher score than the wild type tobacco with each of compound (II) and compound (XII).

Example 48 Obtaining the Present Invention DNA (A16)

(1) Preparation of the chromosomal DNA of Streptomyces ornatus IFO 13069t

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces ornatus IFO 13069t was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A11)

PCR was conducted by utilizing as the template the chromosomal DNA prepared from Streptomyces ornatus IFO 13069t in Example 48(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned into cloning vector pCRII-TOPO (Invitrogen Company). The sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 343 to 1069 of the nucleotide sequence shown in SEQ ID NO: 225 was provided.

Further, the chromosomal DNA prepared in Example 48(1) was digested with restriction enzyme PvuII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide

sequence shown in SEQ ID NO: 265 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 266 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 501 of the nucleotide sequence shown in SEQ ID NO: 235 was provided.

Further, the chromosomal DNA prepared in Example 48(1) was digested with restriction enzyme PvuII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 267 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 268 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1044 to 1454 of the nucleotide sequence shown in SEQ ID NO: 235 was provided.

(3) Sequence analysis of the present invention DNA (A16)

The nucleotide sequence shown in SEQ ID NO: 235 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 48(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 225) consisting of 1251 nucleotides (inclusive of the stop codon) and encoding a 416 amino acid residue (SEQ ID NO: 215) and a nucleotide sequence (SEQ ID NO: 255) consisting of 198 nucleotides (inclusive of the stop codon) and

encoding a 65 amino acid residue (SEQ ID NO: 245). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 215) encoded by the nucleotide sequence shown in SEQ ID NO: 225 was calculated to be 46013Da. Further, the molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 245) encoded by the nucleotide sequence shown in SEQ ID NO: 255 was calculated to be 6768Da.

Example 49 Expression of the Present Invention DNA (A16) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A16)

PCR was conducted by utilizing the GeneAmp High Fidelity PCR System (Applied Biosystems Japan Company) and by utilizing as the template the chromosomal DNA prepared from Streptomyces ornatus IFO 13069t in Example 48(1). As the primers, there was utilized a pairing of the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 269 and the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 286. The PCR reaction solution amounted to 50µl by adding the 2 primers each amounting to 200nM, 50ng of the above chromosomal DNA, 5.0µl of dNTP mix (a mixture of 2.0mM of each of the 4 types of dNTP; Clontech Company), 5.0µl of 10X buffer (containing $MgCl_2$) and 0.5µl of GeneAmp HF enzyme mix and by adding distilled water. The reaction conditions of the PCR were after maintaining 97℃ for 1 minute; repeating 10 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds, and followed by 72℃ for 90 seconds; then conducting 15 cycles of a cycle that included maintaining 97℃ for 15 seconds, followed by 60℃ for 30 seconds and followed by 72℃ for 90seconds (wherein 20 seconds was added to the maintenance at 72℃ for each cycle); and then maintaining 72℃ for 7 minutes. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of

the obtained plasmid DNA was analyzed by utilizing as primers the oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 57, 59, 267, 286 and 288. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 235 was designated as pCR452F. Similarly to Example 32(1), pCR452F was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 235, in which the DNA encoding the present invention protein (A16) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN452F"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN452F.

(2) Expression of the present invention protein (A16) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN452F and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN452F is referred to as "E. coli pKSN452F extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Similarly to Example 32(3), reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. However, as the supernatant fraction, the supernatant fraction prepared in Example 49(2) (E. coli pKSN452F extract or E. coli pKSN2 extract)

was utilized. The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN452F extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 50 Obtaining the Present Invention DNA (A17)

(1) Preparation of the chromosomal DNA of Streptomyces griseus ATCC 10137

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces griseus ATCC 10137 was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A17)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces griseus ATCC 10137 prepared in Example 50(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 343 to 1069 of the nucleotide sequence shown in SEQ ID NO: 226 was provided.

Further, the chromosomal DNA prepared in Example 50(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the

obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 270 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 271 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 361 of the nucleotide sequence shown in SEQ ID NO: 236 was provided.

Further, the chromosomal DNA prepared in Example 50(1) was digested with restriction enzyme PvuII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 272 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 273 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1035 to 1454 of the nucleotide sequence shown in SEQ ID NO: 236 was provided.

(3) Sequence analysis of the present invention DNA (A17)

The nucleotide sequence shown in SEQ ID NO: 236 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 50(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 226) consisting of 1251 nucleotides (inclusive of the stop codon) and encoding a 416 amino acid residue (SEQ ID NO: 216) and a nucleotide

sequence (SEQ ID NO: 256) consisting of 198 nucleotides (inclusive of the stop codon) and encoding a 65 amino acid residue (SEQ ID NO: 246). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 216) encoded by the nucleotide sequence shown in SEQ ID NO: 226 was calculated to be 46082Da. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 246) encoded by the nucleotide sequence shown in SEQ ID NO: 256 was calculated to be 6768Da. The nucleotide sequence shown in SEQ ID NO: 256 is 100% identical to the nucleotide sequence shown in SEQ ID NO: 255. The amino acid sequence shown in SEQ ID NO: 246 is 100% identical to the amino acid sequence shown in SEQ ID NO: 245.

Example 51 Expression of the Present Invention DNA (A17) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A17)

PCR was conducted similarly to Example 32(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces griseus ATCC 10137 in Example 50(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 274 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 275. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was sequenced by utilizing as primers the oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 57, 59, 274, 276 and 277. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 236 was designated as pCR608F. Similarly to Example 32(1), pCR608F was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated

to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 236, in which the DNA encoding the present invention protein (A17) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN608F"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN608F.

(2) Expression of the present invention protein (A17) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN608F and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN608F is referred to as "E. coli pKSN608F extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Similarly to Example 32(3), reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. However, as the supernatant fraction, the supernatant fraction prepared in Example 51(2) (E. coli pKSN608F extract or E. coli pKSN2 extract) was utilized. The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN608F extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 52 Obtaining the Present Invention DNA (A18)

(1) Preparation of the chromosomal DNA of Streptomyces achromogenes IFO 12735

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces achromogenes IFO 12735 was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A18)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces achromogenes IFO 12735 prepared in Example 52(1) and by utilizing primer pairing 17, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 526 to 1048 of the nucleotide sequence shown in SEQ ID NO: 227 was provided.

Further, the chromosomal DNA prepared in Example 52(1) was digested with restriction enzyme HincII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 278 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 279 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The

nucleotide sequence shown in nucleotides 1 to 600 of the nucleotide sequence shown in SEQ ID NO: 237 was provided.

Further, the chromosomal DNA prepared in Example 52(1) was digested with restriction enzyme BalI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 163 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 164 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 983 to 1449 of the nucleotide sequence shown in SEQ ID NO: 237 was provided.

(3) Sequence analysis of the present invention DNA (A18)

The nucleotide sequence shown in SEQ ID NO: 237 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 52(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 227) consisting of 1230 nucleotides (inclusive of the stop codon) and encoding a 409 amino acid residue (SEQ ID NO: 217) and a nucleotide sequence (SEQ ID NO: 257) consisting of 207 nucleotides (inclusive of the stop codon) and encoding a 68 amino acid residue (SEQ ID NO: 247). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 217) encoded by the nucleotide sequence shown in SEQ ID NO: 227 was calculated to be 45099Da. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 247) encoded by the

nucleotide sequence shown in SEQ ID NO: 257 was calculated to be 7193Da.

Example 53 Expression of the Present Invention DNA (A18) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A18)

PCR was conducted similarly to Example 49(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces achromogenes IFO 12735 in Example 52(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 183 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 280. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was analyzed by utilizing as primers the oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 67, 68, 163, 279 and 281. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 237 was designated as pCR646BF. Similarly to Example 32(1), pCR646BF was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 237, in which the DNA encoding the present invention protein (A18) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN646BF"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN646BF.

(2) Expression of the present invention protein (A18) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN464BF and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN646BF is referred to as "E. coli pKSN646BF extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Similarly to Example 32(3), reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. However, as the supernatant fraction, the supernatant fraction prepared in Example 53(2) (E. coli pKSN646BF extract or E. coli pKSN2 extract) was utilized. The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN646BF extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 54 Obtaining the Present Invention DNA (A19)

(1) Preparation of the chromosomal DNA of Streptomyces griseus IFO 13849T

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces griseus IFO 13849T was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present

invention DNA (A19)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces griseus IFO 13849T prepared in Example 54(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 343 to 1069 of the nucleotide sequence shown in SEQ ID NO: 228 was provided.

Further, the chromosomal DNA prepared in Example 54(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 282 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 283 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 358 of the nucleotide sequence shown in SEQ ID NO: 238 was provided.

Further, the chromosomal DNA prepared in Example 54(1) was digested with restriction enzyme HincII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 284 and primer AP1. Next, PCR was conducted under the

conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 285 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1005 to 1454 of the nucleotide sequence shown in SEQ ID NO: 238 was provided.

(3) Sequence analysis of the present invention DNA (A19)

The nucleotide sequence shown in SEQ ID NO: 238 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 54(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 228) consisting of 1251 nucleotides (inclusive of the stop codon) and encoding a 416 amino acid residue (SEQ ID NO: 218) and a nucleotide sequence (SEQ ID NO: 258) consisting of 156 nucleotides (inclusive of the stop codon) and encoding a 51 amino acid residue (SEQ ID NO: 248). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 218) encoded by the nucleotide sequence shown in SEQ ID NO: 228 was calculated to be 45903Da. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 248) encoded by the nucleotide sequence shown in SEQ ID NO: 258 was calculated to be 5175Da.

Example 55 Expression of the Present Invention DNA (A19) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A19)

PCR was conducted similarly to Example 49(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces griseus IFO 13849T in Example 54(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 286 and an oligonucleotide having the nucleotide sequence shown

in SEQ ID NO: 287. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was analyzed by utilizing as primers the oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 57, 59, 284, 286 and 288. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 238 was designated as pCR1502F. Similarly to Example 32(1), pCR1502F was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 238, in which the DNA encoding the present invention protein (A19) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN1502F"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN1502F.

(2) Expression of the present invention protein (A18) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN1502F and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN1502F is referred to as "E. coli pKSN1502F extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Similarly to Example 32(3), reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. However, as the supernatant fraction, the supernatant fraction prepared in Example 55(2) (E. coli pKSN1502F extract or E. coli pKSN2 extract) was utilized. The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN1502F extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 56 Obtaining the Present Invention DNA (A20)

(1) Preparation of the chromosomal DNA of Streptomyces lanatus IFO 12787T

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces lanatus IFO 12787T was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A20)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces lanatus IFO 12787T prepared in Example 56(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 304 to 1036 of the nucleotide sequence shown in SEQ ID NO: 229 was provided.

Further, the chromosomal DNA prepared in Example 56(1) was digested with restriction enzyme PmacI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 278 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 289 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 318 of the nucleotide sequence shown in SEQ ID NO: 239 was provided.

Further, the chromosomal DNA prepared in Example 56(1) was digested with restriction enzyme StuI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 290 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 291 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 969 to 1461 of the nucleotide sequence shown in SEQ ID NO: 239 was provided.

(3) Sequence analysis of the present invention DNA (A20)

The nucleotide sequence shown in SEQ ID NO: 239 was obtained by connecting the

nucleotide sequences provided by the DNA obtained in Example 56(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 229) consisting of 1218 nucleotides (inclusive of the stop codon) and encoding a 405 amino acid residue (SEQ ID NO: 219) and a nucleotide sequence (SEQ ID NO: 259) consisting of 231 nucleotides (inclusive of the stop codon) and encoding a 76 amino acid residue (SEQ ID NO: 249). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 219) encoded by the nucleotide sequence shown in SEQ ID NO: 229 was calculated to be 45071Da. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 249) encoded by the nucleotide sequence shown in SEQ ID NO: 259 was calculated to be 7816Da.

Example 57 Expression of the Present Invention DNA (A20) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A20)

PCR was conducted similarly to Example 49(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces lanatus IFO 12787T in Example 56(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 292 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 293. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was analyzed by utilizing as primers the oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 67, 68, 188, 278 and 290. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 239 was designated as pCR1525F. Similarly to Example 32(1), pCR1525F was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion

products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 239, in which the DNA encoding the present invention protein (A20) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN1525F"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN1525F.

(2) Expression of the present invention protein (A20) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN1525F and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN1525F is referred to as "E. coli pKSN1525F extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Similarly to Example 32(3), reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. However, as the supernatant fraction, the supernatant fraction prepared in Example 57(2) (E. coli pKSN1525F extract or E. coli pKSN2 extract) was utilized. The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was

detected from the reaction solution containing E. coli pKSN1525F extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 58 Obtaining the Present Invention DNA (A21)

(1) Preparation of the chromosomal DNA of Streptomyces misawanensis IFO 13855T

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces misawanensis IFO 13855T was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A21)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces misawanensis IFO 13855T prepared in Example 58(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 328 to 1063 of the nucleotide sequence shown in SEQ ID NO: 230 was provided.

Further, the chromosomal DNA prepared in Example 58(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 294 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template

and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 295 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 341 of the nucleotide sequence shown in SEQ ID NO: 240 was provided.

Further, the chromosomal DNA prepared in Example 58(1) was digested with restriction enzyme HincII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 296 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 297 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1017 to 1458 of the nucleotide sequence shown in SEQ ID NO: 240 was provided.

(3) Sequence analysis of the present invention DNA (A21)

The nucleotide sequence shown in SEQ ID NO: 240 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 58(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 230) consisting of 1245 nucleotides (inclusive of the stop codon) and encoding a 414 amino acid residue (SEQ ID NO: 220) and a nucleotide sequence (SEQ ID NO: 260) consisting of 201 nucleotides (inclusive of the stop codon) and encoding a 66 amino acid residue (SEQ ID NO: 250). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 220) encoded by the nucleotide

sequence shown in SEQ ID NO: 230 was calculated to be 45806Da. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 250) encoded by the nucleotide sequence shown in SEQ ID NO: 260 was calculated to be 6712Da.

Example 59 Expression of the Present Invention DNA (A21) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A21)

PCR was conducted similarly to Example 32(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces misawanensis IFO 13855T in Example 58(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 298 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 299. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was analyzed by utilizing as primers the oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 57, 59, 296, 298 and 300. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 240 was designated as pCR1543BF. Similarly to Example 32(1), pCR1543BF was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 240, in which the DNA encoding the present invention protein (A21) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN1543BF"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN1543BF.

(2) **Expression of the present invention protein (A21) in E. coli and recovery of said protein**

Similarly to Example 4(2), each of E. coli JM109/pKSN1543BF and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN1543BF is referred to as "E. coli pKSN1543BF extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) **Detection of the ability to convert compound (II) to compound (III)**

Similarly to Example 32(3), reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. However, as the supernatant fraction, the supernatant fraction prepared in Example 59(2) (E. coli pKSN1543BF extract or E. coli pKSN2 extract) was utilized. The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN1543BF extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 60 Obtaining the Present Invention DNA (A22)

(1) **Preparation of the chromosomal DNA of Streptomyces pallidus IFO 13434T**

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces pallidus IFO 13434T was prepared.

(2) **Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A22)**

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces pallidus IFO 13434T prepared in Example 60(1) and by utilizing primer pairing 15, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 483 to 1048 of the nucleotide sequence shown in SEQ ID NO: 231 was provided.

Further, the chromosomal DNA prepared in Example 60(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 301 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 302 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 68 to 516 of the nucleotide sequence shown in SEQ ID NO: 241 was provided.

Further, the chromosomal DNA prepared in Example 60(1) was digested with restriction enzyme HincII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the

obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 302 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 303 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 270 of the nucleotide sequence shown in SEQ ID NO: 241 was provided.

Further, the chromosomal DNA prepared in Example 60(1) was digested with restriction enzyme HincII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 304 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 305 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 982 to 1448 of the nucleotide sequence shown in SEQ ID NO: 241 was provided.

(3) **Sequence analysis of the present invention DNA (A22)**

The nucleotide sequence shown in SEQ ID NO: 241 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 60(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 231) consisting of 1230 nucleotides (inclusive of the stop codon) and encoding a 409 amino acid residue (SEQ ID NO: 221) and a nucleotide

sequence (SEQ ID NO: 261) consisting of 195 nucleotides (inclusive of the stop codon) and encoding a 64 amino acid residue (SEQ ID NO: 251). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 221) encoded by the nucleotide sequence shown in SEQ ID NO: 231 was calculated to be 45050Da. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 251) encoded by the nucleotide sequence shown in SEQ ID NO; 261 was calculated to be 6914Da.

Example 61 Expression of the Present Invention DNA (A22) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A22)

PCR was conducted similarly to Example 32(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces pallidus IFO 13434T in Example 60(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 306 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 307. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was analyzed by utilizing as primers the oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 67, 68 and 308. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 241 was designated as pCR1558BF. Similarly to Example 32(1), pCR1558BF was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 241, in which the DNA encoding the present invention protein (A22) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as

"pKSN1558BF"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN1558BF.

(2) Expression of the present invention protein (A22) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN1558BF and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN1558BF is referred to as "E. coli pKSN1558BF extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Similarly to Example 32(3), reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. However, as the supernatant fraction, the supernatant fraction prepared in Example 61(2) (E. coli pKSN1558BF extract or E. coli pKSN2 extract) was utilized. The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN1558BF extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 62 Obtaining the Present Invention DNA (A23)

(1) Preparation of the chromosomal DNA of Streptomyces roseorubens IFO 13682T

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces roseorubens IFO 13682T was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A23)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces roseorubens IFO 13682T prepared in Example 62(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 289 to 1015 of the nucleotide sequence shown in SEQ ID NO: 232 was provided.

Further, the chromosomal DNA prepared in Example 62(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 309 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 310 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 354 of the nucleotide sequence shown in SEQ ID NO: 242 was provided.

Further, the chromosomal DNA prepared in Example 62(1) was digested with restriction enzyme PvuII. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 311 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 312 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 966 to 1411 of the nucleotide sequence shown in SEQ ID NO: 242 was provided.

(3) Sequence analysis of the present invention DNA (A23)

The nucleotide sequence shown in SEQ ID NO: 242 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 62(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 232) consisting of 1197 nucleotides (inclusive of the stop codon) and encoding a 398 amino acid residue (SEQ ID NO: 222) and a nucleotide sequence (SEQ ID NO: 262) consisting of 201 nucleotides (inclusive of the stop codon) and encoding a 66 amino acid residue (SEQ ID NO: 252). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 222) encoded by the nucleotide sequence shown in SEQ ID NO: 232 was calculated to be 43624Da. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 252) encoded by the nucleotide sequence shown in SEQ ID NO: 262 was calculated to be 6797Da.

Example 63 Expression of the Present Invention DNA (A23) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A23)

PCR was conducted similarly to Example 49(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces roseorubens IFO 13682T in Example 62(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 313 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 314. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was analyzed by utilizing as primers the oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 67, 68, 309, 311 and 315. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 242 was designated as pCR1584F. Similarly to Example 32(1), pCR1584F was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 242, in which the DNA encoding the present invention protein (A23) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN1584F"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN1584F.

(2) Expression of the present invention protein (A23) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN1584F and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were

prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN1584F is referred to as "E. coli pKSN1584F extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Similarly to Example 32(3), reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. However, as the supernatant fraction, the supernatant fraction prepared in Example 63(2) (E. coli pKSN1584F extract or E. coli pKSN2 extract) was utilized. The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN1584F extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 64 Obtaining the Present Invention DNA (A24)

(1) Preparation of the chromosomal DNA of Streptomyces rutgersensis IFO 15875T

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces rutgersensis IFO 15875T was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A24)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces rutgersensis IFO 15875T prepared in Example 64(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 322 to 1057 of the nucleotide sequence shown in SEQ ID NO: 233 was provided.

Further, the chromosomal DNA prepared in Example 64(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 316 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 317 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 384 of the nucleotide sequence shown in SEQ ID NO: 243 was provided.

Further, the chromosomal DNA prepared in Example 64(1) was digested with restriction enzyme NaeI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 318 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template

and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 319 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 992 to 1466 of the nucleotide sequence shown in SEQ ID NO: 243 was provided.

(3) Sequence analysis of the present invention DNA (A24)

The nucleotide sequence shown in SEQ ID NO: 243 was obtained by connecting the nucleotide sequences provided by the DNA obtained in Example 64(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 233) consisting of 1245 nucleotides (inclusive of the stop codon) and encoding a 414 amino acid residue (SEQ ID NO: 223) and a nucleotide sequence (SEQ ID NO: 263) consisting of 198 nucleotides (inclusive of the stop codon) and encoding a 65 amino acid residue (SEQ ID NO: 253). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 223) encoded by the nucleotide sequence shown in SEQ ID NO: 233 was calculated to be 45830Da. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 253) encoded by the nucleotide sequence shown in SEQ ID NO: 263 was calculated to be 7034Da.

Example 65 Expression of the Present Invention DNA (A24) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A24)

PCR was conducted similarly to Example 49(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces rutgersensis IFO 15875T in Example 64(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 320 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 321. Similarly to Example 32(1), the DNA was purified

from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was sequenced by utilizing as primers the oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 67, 68 and 322. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 243 was designated as pCR1589BF. Similarly to Example 32(1), pCR1589BF was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 243, in which the DNA encoding the present invention protein (A24) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN1589BF"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN1589BF.

(2) Expression of the present invention protein (A24) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN1589BF and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN1589BF is referred to as "E. coli pKSN1589BF extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Similarly to Example 32(3), reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. However, as the supernatant fraction, the supernatant fraction prepared in Example 65(2) (E. coli pKSN1589BF extract or E. coli pKSN2 extract) was utilized. The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was detected from the reaction solution containing E. coli pKSN1589BF extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 66 Obtaining the Present Invention DNA (A25)

(1) Preparation of the chromosomal DNA of Streptomyces steffisburgensis IFO 13446T

Under the method described in Example 31(1), the chromosomal DNA of Streptomyces steffisburgensis IFO 13446T was prepared.

(2) Isolation of DNA having a partial nucleotide sequence of the present invention DNA (A25)

PCR was conducted by utilizing as the template the chromosomal DNA of Streptomyces steffisburgensis IFO 13446T prepared in Example 66(1) and by utilizing primer pairing 14, in accordance with the method described in Example 29. Similarly to Example 31(2), the amplified DNA was cloned to cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence thereof was analyzed. As a result, the nucleotide sequence shown in nucleotides 289 to 1015 of the nucleotide sequence shown in SEQ ID NO: 234 was provided.

Further, the chromosomal DNA prepared in Example 66(1) was digested with restriction enzyme SmaI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 323 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 324 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 1 to 303 of the nucleotide sequence shown in SEQ ID NO: 244 was provided.

Further, the chromosomal DNA prepared in Example 66(1) was digested with restriction enzyme PmacI. A genome walker library was produced by utilizing the obtained DNA, according to the method described in Example 26(3). PCR was conducted under the conditions described in Example 26(3) to obtain the first PCR products, by utilizing the obtained library as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 311 and primer AP1. Next, PCR was conducted under the conditions described in Example 26(3), by utilizing the first PCR products as the template and by utilizing the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 325 and primer AP2. The nucleotide sequence of the obtained DNA was analyzed. The nucleotide sequence shown in nucleotides 966 to 1411 of the nucleotide sequence shown in SEQ ID NO: 244 was provided.

(3) Sequence analysis of the present invention DNA (A25)

The nucleotide sequence shown in SEQ ID NO: 244 was obtained by connecting the

nucleotide sequences provided by the DNA obtained in Example 66(2). Two open reading frames (ORF) were present in said nucleotide sequence. As such, there was contained a nucleotide sequence (SEQ ID NO: 234) consisting of 1197 nucleotides (inclusive of the stop codon) and encoding a 398 amino acid residue (SEQ ID NO: 224) and a nucleotide sequence (SEQ ID NO: 264) consisting of 201 nucleotides (inclusive of the stop codon) and encoding a 66 amino acid residue (SEQ ID NO: 254). The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 224) encoded by the nucleotide sequence shown in SEQ ID NO: 234 was calculated to be 44175Da. The molecular weight of the protein consisting of the amino acid sequence (SEQ ID NO: 254) encoded by the nucleotide sequence shown in SEQ ID NO: 264 was calculated to be 6685Da.

Example 67 Expression of the Present Invention DNA (A25) in E. Coli

(1) Production of a transformed E. coli having the present invention DNA (A25)

PCR was conducted similarly to Example 49(1), other than utilizing as a template the chromosomal DNA prepared from Streptomyces steffisburgensis IFO 13446T in Example 66(1) and utilizing as the primers the oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 326 and an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 327. Similarly to Example 32(1), the DNA was purified from the reaction solution of PCR and cloned into the cloning vector pCRII-TOPO (Invitrogen Company). The nucleotide sequence of the obtained plasmid DNA was sequenced by utilizing as primers the oligonucleotides having the nucleotide sequences shown, respectively, in SEQ ID NOs: 67, 68, 311, 315 and 323. Based on the obtained results, the plasmid having the nucleotide sequence shown in SEQ ID NO: 244 was designated as pCR1609F. Similarly to Example 32(1), pCR1609F was digested with restriction enzymes NdeI and HindIII. A DNA of about 1.5kbp was purified from the

digestion products. The obtained DNA and the plasmid pKSN2 digested with NdeI and HindIII were ligated to obtain a plasmid containing the nucleotide sequence shown in SEQ ID NO: 244, in which the DNA encoding the present invention protein (A25) is inserted between the NdeI site and the HindIII site of pKSN2 (hereinafter referred to as "pKSN1609F"). Said plasmid was introduced into E. Coli JM109. The obtained E. coli transformant was designated JM109/pKSN1609F.

(2) Expression of the present invention protein (A25) in E. coli and recovery of said protein

Similarly to Example 4(2), each of E. coli JM109/pKSN1609F and JM109/pKSN2 was cultured. The cells were recovered. Cell lysate solutions were prepared. Under the method described in Example 4(2), supernatant fractions were prepared from the cell lysate solutions (hereinafter, the supernatant fraction obtained from E. coli JM109/pKSN1609F is referred to as "E. coli pKSN1609F extract " and the supernatant fraction obtained from E. coli JM109/pKSN2 is referred to as "E. coli pKSN2 extract ").

(3) Detection of the ability to convert compound (II) to compound (III)

Similarly to Example 32(3), reaction solutions of 30μl were prepared and maintained for 10 minutes at 30℃. However, as the supernatant fraction, the supernatant fraction prepared in Example 67(2) (E. coli pKSN1609F extract or E. coli pKSN2 extract) was utilized. The reaction solutions after the maintenance were extracted with ethyl acetate and the extracted layers were TLC analyzed. After developing the TLC plate, the presence of a spot thereon corresponding to compound (III) labeled with ^{14}C were examined (Rf value 0.24 and 0.29). A spot corresponding to compound (III) was

detected from the reaction solution containing E. coli pKSN1609F extract. In contrast, such a spot was not detected from the reaction solution containing E. coli pKSN2 extract.

Example 68 Metabolism of Compounds by the Present Invention Protein (A16), (A17), (A18), (A19), (A20), (A21), (A22), (A23), (A24) or (A25)

(1) Metabolism of compound (XII) by the present invention protein (A16)

There was prepared 100μl of a reaction solution of 50mM potassium phosphate buffer (pH7.0) containing 12.5ppm of compound (XII), 3mM of β-NADPH (hereinafter, referred to as "component A") (Oriental Yeast Company), 1mg/ml of a ferredoxin derived from spinach (hereinafter referred to as "component B") (Sigma Company), 0.15U/ml of ferredoxin reductase (hereinafter, referred to as "component C") (Sigma Company) and 20μl of the supernatant fraction recovered in Example 49(2). The reaction solution was maintained at 30℃ for 10 minutes. Further, there was prepared and maintained similarly 100μl of a reaction solution of a 50mM potassium phosphate buffer (pH 7.0) having no addition of at least one component utilized in the composition of the above reaction solution, selected from component A, component B, component C and the supernatant fraction prepared in Example 49(2). Five microliters (5μl) of 2N HCl and 100μl of ethyl acetate were added and mixed into each of the reaction solutions after the maintenance. The supernatant centrifuged at 8,000xg was filtered with UltraFree MC 0.22μm filter unit (Millipore Company). Forty microliters (40μl) of the liquid filtrate (hereinafter, the liquid filtrate derived from the reaction solution containing component A, component B, component C and 20μl of supernatant fraction recovered in Example 49(2) is referred to as "(XII) metabolism solution (A16)"; further, the liquid filtrate derived from the reaction solution containing no component A, no component B, no component C and no supernatant fraction recovered in Example 49(2) is referred to as "(XII) control solution

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CLAIMS

1. A DNA encoding a herbicide metabolizing protein, wherein said protein is selected from the group consisting of:

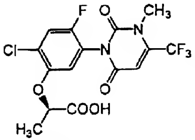
(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

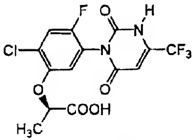
(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II):



(II)

to a compound of formula (III):



(III)

and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula

(III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid

sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224;

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising the nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis, Streptomyces steffisburgensis or Saccharopolyspora taberi.

2. A DNA comprising a nucleotide sequence selected from the group consisting of:

(a1) the nucleotide sequence shown in SEQ ID NO: 6;

(a2) the nucleotide sequence shown in SEQ ID NO: 7;

(a3) the nucleotide sequence shown in SEQ ID NO: 8;

(a4) the nucleotide sequence shown in SEQ ID NO: 109;

(a5) the nucleotide sequence shown in SEQ ID NO: 139;

(a6) the nucleotide sequence shown in SEQ ID NO: 140;

(a7) the nucleotide sequence shown in SEQ ID NO: 141;

(a8) the nucleotide sequence shown in SEQ ID NO: 142;

(a9) the nucleotide sequence shown in SEQ ID NO: 143;

(a10) the nucleotide sequence shown in SEQ ID NO: 225;

(a11) the nucleotide sequence shown in SEQ ID NO: 226;

(a12) the nucleotide sequence shown in SEQ ID NO: 227;

(a13) the nucleotide sequence shown in SEQ ID NO: 228;

(a14) the nucleotide sequence shown in SEQ ID NO: 229;

(a15) the nucleotide sequence shown in SEQ ID NO: 230;

(a16) the nucleotide sequence shown in SEQ ID NO: 231;

(a17) the nucleotide sequence shown in SEQ ID NO: 232;

(a18) the nucleotide sequence shown in SEQ ID NO: 233;

(a19) the nucleotide sequence shown in SEQ ID NO: 234;

(a20) a nucleotide sequence encoding an amino acid sequence of a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), said nucleotide sequence having at least 80% sequence identity with a nucleotide

sequence shown in any one of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 109; and

(a21) a nucleotide sequence encoding an amino acid sequence of a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), said nucleotide sequence having at least 90% sequence identity with a nucleotide sequence shown in any one of SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 225, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233 or SEQ ID NO: 234.

3. The DNA according to claim 1, comprising a nucleotide sequence encoding an amino acid sequence of said protein, wherein the codon usage in said nucleotide sequence is within the range of plus or minus 4% of the codon usage in genes from the species of a host cell to which the DNA is introduced and the GC content of said nucleotide sequence is at least 40% and at most 60%.

4. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 214.

5. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 368.

6. A DNA comprising the nucleotide sequence shown in SEQ ID NO: 393.

7. A DNA in which a DNA having a nucleotide sequence encoding an intracellular organelle transit signal sequence is linked upstream of the DNA according to

claim 1 in frame.

8. A DNA in which the DNA according to claim 1 and a promoter functional in a host cell are operably linked.

9. A vector comprising the DNA according to claim 1.

10. A method of producing a vector comprising a step of inserting the DNA according to claim 1 into a vector replicable in a host cell.

11. A transformant in which the DNA according to claim 1 is introduced into a host cell.

12. The transformant according to claim 11, wherein the host cell is a microorganism cell or a plant cell.

13. A method of producing a transformant comprising a step of introducing into a host cell, the DNA according to claim 1.

14. A method of producing a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method comprising a steps of culturing the transformant according to claim 11 and recovering the produced said protein.

15. Use of the DNA according to claim 1 for producing a protein having the ability to convert a compound of formula (II) to a compound of formula (III).

16. A method of giving a plant resistance to a herbicide, said method comprising a step of introducing into and expressing in a plant cell, the DNA according to claim 1.

17. A polynucleotide having a partial nucleotide sequence of a DNA according to claim 1 or a nucleotide sequence complimentary to said partial nucleotide sequence.

18. A method of detecting a DNA encoding a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method comprising a step of detecting a DNA to which a probe is hybridized in a hybridization using as the probe the DNA according to claim 1 or the polynucleotide according to claim 17.

19. A method of detecting a DNA encoding a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method comprising a step of detecting a DNA amplified in a polymerase chain reaction with the polynucleotide according to claim 17 as a primer.

20. The method according to claim 19, wherein at least one of the primers is selected from the group consisting of a polynucleotide comprising the nucleotide sequence shown in any one of SEQ ID NOs:124 to 128 and a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 129.

21. A method of obtaining a DNA encoding a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method

comprising a step of recovering the DNA detected by the method according to claim 18 or 19.

22. A method of screening a cell having a DNA encoding a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method comprising a step of detecting said DNA from a test cell by the method according to claim 18 or 19.

23. A herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224;

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of

formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising the nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis, Streptomyces steffisburgensis or Saccharopolyspora taberi.

24. An antibody recognizing a herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224;

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising the nucleotide

sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis, Streptomyces steffisburgensis or Saccharopolyspora taberi.

25. A method of detecting a herbicide metabolizing protein, said method comprising:

(3) a step of contacting a test substance with an antibody recognizing said protein and

(4) a step of detecting a complex of said protein and said antibody, arising from said contact,

wherein said protein is selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2,

SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence

identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224;

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising the nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus,

Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis, Streptomyces steffisburgensis or Saccharopolyspora taberi.

26. An analysis or detection kit comprising the antibody according to claim 24.

27. A DNA encoding a ferredoxin selected from the group consisting of:

(B1) a protein comprising an amino acid sequence shown in SEQ ID NO: 12;

(B2) a protein comprising an amino acid sequence shown in SEQ ID NO: 13;

(B3) a protein comprising an amino acid sequence shown in SEQ ID NO: 14;

(B4) a protein comprising an amino acid sequence shown in SEQ ID NO: 111;

(B5) a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

(B6) a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

(B7) a protein comprising an amino acid sequence shown in SEQ ID NO: 149;

(B8) a protein comprising an amino acid sequence shown in SEQ ID NO: 150;

(B9) a protein comprising an amino acid sequence shown in SEQ ID NO: 151;

(B10) a protein comprising an amino acid sequence shown in SEQ ID NO: 152;

(B11) a protein comprising an amino acid sequence shown in SEQ ID NO: 153;

(B12) a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 149, SEQ

ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, or SEQ ID NO: 253 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 150, SEQ ID NO: 252 or SEQ ID NO: 254;

(B13) a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253 or SEQ ID NO: 254;

(B14) a protein comprising the amino acid sequence shown in SEQ ID NO: 245;

(B15) a protein comprising the amino acid sequence shown in SEQ ID NO: 247;

(B16) a protein comprising the amino acid sequence shown in SEQ ID NO: 248;

(B17) a protein comprising the amino acid sequence shown in SEQ ID NO: 249;

(B18) a protein comprising the amino acid sequence shown in SEQ ID NO: 250;

(B19) a protein comprising the amino acid sequence shown in SEQ ID NO: 251;

(B20) a protein comprising the amino acid sequence shown in SEQ ID NO: 252;

(B21) a protein comprising the amino acid sequence shown in SEQ ID NO: 253; and

(B22) a protein comprising the amino acid sequence shown in SEQ ID NO: 254.

28. A DNA comprising a nucleotide sequence selected from the group consisting of:

(b1) a nucleotide sequence shown in SEQ ID NO: 15;

(b2) a nucleotide sequence shown in SEQ ID NO: 16;

(b3) a nucleotide sequence shown in SEQ ID NO: 17;

(b4) a nucleotide sequence shown in SEQ ID NO: 112;

(b5) a nucleotide sequence shown in SEQ ID NO: 154;

(b6) a nucleotide sequence shown in SEQ ID NO: 155;

(b7) a nucleotide sequence shown in SEQ ID NO: 156;

(b8) a nucleotide sequence shown in SEQ ID NO: 157;

(b9) a nucleotide sequence shown in SEQ ID NO: 158;

(b10) a nucleotide sequence shown in SEQ ID NO: 255;

(b11) a nucleotide sequence shown in SEQ ID NO: 257;

(b12) a nucleotide sequence shown in SEQ ID NO: 258;

(b13) a nucleotide sequence shown in SEQ ID NO: 259;

(b14) a nucleotide sequence shown in SEQ ID NO: 260;

(b15) a nucleotide sequence shown in SEQ ID NO: 261;

(b16) a nucleotide sequence shown in SEQ ID NO: 262;

(b17) a nucleotide sequence shown in SEQ ID NO: 263;

(b18) a nucleotide sequence shown in SEQ ID NO: 264; and

(b19) a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence shown in any one of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 112, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263 or SEQ ID NO: 264.

29. A vector comprising a DNA according to claim 28.

30. A transformant in which the DNA according to claim 28 is introduced into a host cell.

31. A ferredoxin selected from the group consisting of:

(B1) a protein comprising an amino acid sequence shown in SEQ ID NO: 12;

(B2) a protein comprising an amino acid sequence shown in SEQ ID NO: 13;

(B3) a protein comprising an amino acid sequence shown in SEQ ID NO: 14;

(B4) a protein comprising an amino acid sequence shown in SEQ ID NO: 111;

(B5) a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

(B6) a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO 14 or SEQ ID NO: 111;

(B7) a protein comprising an amino acid sequence shown in SEQ ID NO: 149;

(B8) a protein comprising an amino acid sequence shown in SEQ ID NO: 150;

(B9) a protein comprising an amino acid sequence shown in SEQ ID NO: 151;

(B10) a protein comprising an amino acid sequence shown in SEQ ID NO: 152;

(B11) a protein comprising an amino acid sequence shown in SEQ ID NO: 153;

(B12) a ferredoxin comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, or SEQ ID NO: 253 or an amino acid sequence having at least 90% sequence identity

with an amino acid sequence shown in any one of SEQ ID NO: 150, SEQ ID NO: 252 or SEQ ID NO: 254;

(B13) a ferredoxin comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253 or SEQ ID NO: 254;

(B14) a protein comprising the amino acid sequence shown in SEQ ID NO: 245;

(B15) a protein comprising the amino acid sequence shown in SEQ ID NO: 247;

(B16) a protein comprising the amino acid sequence shown in SEQ ID NO: 248;

(B17) a protein comprising the amino acid sequence shown in SEQ ID NO: 249;

(B18) a protein comprising the amino acid sequence shown in SEQ ID NO: 250;

(B19) a protein comprising the amino acid sequence shown in SEQ ID NO: 251;

(B20) a protein comprising the amino acid sequence shown in SEQ ID NO: 252;

(B21) a protein comprising the amino acid sequence shown in SEQ ID NO: 253; and

(B22) a protein comprising the amino acid sequence shown in SEQ ID NO: 254.

32. A DNA comprising a nucleotide sequence selected from the group consisting of:

(ab1) a nucleotide sequence shown in SEQ ID NO: 9;

(ab2) a nucleotide sequence shown in SEQ ID NO: 10;

(ab3) a nucleotide sequence shown in SEQ ID NO: 11;

(ab4) a nucleotide sequence shown in SEQ ID NO: 110;

(ab5) a nucleotide sequence shown in SEQ ID NO: 144;

(ab6) a nucleotide sequence shown in SEQ ID NO: 145;

(ab7) a nucleotide sequence shown in SEQ ID NO: 146;

(ab8) a nucleotide sequence shown in SEQ ID NO: 147;

(ab9) a nucleotide sequence shown in SEQ ID NO: 148;

(ab10) a nucleotide sequence shown in SEQ ID NO: 235;

(ab11) a nucleotide sequence shown in SEQ ID NO: 236;

(ab12) a nucleotide sequence shown in SEQ ID NO: 237;

(ab13) a nucleotide sequence shown in SEQ ID NO: 238;

(ab14) a nucleotide sequence shown in SEQ ID NO: 239;

(ab15) a nucleotide sequence shown in SEQ ID NO: 240;

(ab16) a nucleotide sequence shown in SEQ ID NO: 241;

(ab17) a nucleotide sequence shown in SEQ ID NO: 242;

(ab18) a nucleotide sequence shown in SEQ ID NO: 243; and

(ab19) a nucleotide sequence shown in SEQ ID NO: 244.

33. A vector comprising the DNA according to claim 32.

34. A transformant in which the DNA according to claim 32 is introduced into a host cell.

35. The transformant according to claim 34, wherein the host cell is a microorganism cell or a plant cell.

36. A method of producing a transformant comprising a step of introducing into a host cell the DNA according to claim 32.

37. A method of producing a protein having the ability to convert a compound of formula (II) to a compound of formula (III), said method comprising a step of culturing the transformant according to claim 34 and recovering the produced said protein.

38. A method of controlling weeds comprising a step of applying a compound to a cultivation area of a plant expressing at least one herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of

formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

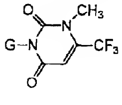
(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

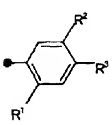
(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224,

wherein said compound is a compound of formula (I):

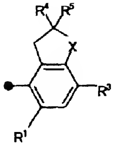


(I)

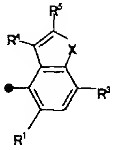
wherein in formula (I) G represents a group shown in any one of the following G-1 to G-9:



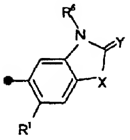
G-1



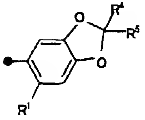
G-2



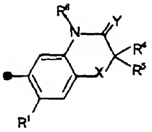
G-3



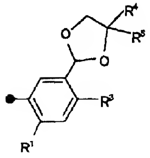
G-4



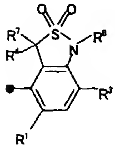
G-5



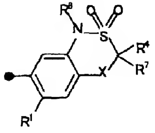
G-6



G-7



G-8



G-9

wherein in G-1 to G-9,

X represents an oxygen atom or sulfur atom;

Y represents an oxygen atom or sulfur atom;

R^1 represents a hydrogen atom or halogen atom;

R^2 represents a hydrogen atom, C_1-C_8 alkyl group, C_1-C_8 haloalkyl group, halogen atom, hydroxyl group, $-OR^9$ group, -SH group, $-S(O)pR^9$ group, $-COR^9$ group, $-CO_2R^9$ group, $-C(O)SR^9$ group, $-C(O)NR^{11}R^{12}$ group, $-CONH_2$ group, -CHO group, $-CR^9{=}NOR^{18}$ group, $-CH{=}CR^{19}CO_2R^9$ group, $-CH_2CHR^{19}CO_2R^9$ group, $-CO_2N{=}CR^{13}R^{14}$ group, nitro group, cyano group, $-NHSO_2R^{15}$ group, $-NHSO_2NHR^{15}$ group, $-NR^9R^{20}$ group, $-NH_2$ group or phenyl group that may be substituted with one or more C_1-C_4 alkyl groups which may be the same or different;

p represents 0, 1 or 2;

R^3 represents C_1-C_2 alkyl group, C_1-C_2 haloalkyl group, $-OCH_3$ group, $-SCH_3$ group, $-OCHF_2$ group, halogen atom, cyano group, nitro group or C_1-C_3 alkoxy group substituted with a phenyl group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_3 alkyl group, C_1-C_3 haloalkyl group, OR^{28} group, $NR^{11}R^{28}$ group, SR^{28} group, cyano group, CO_2R^{28} group and nitro group;

R^4 represents a hydrogen atom, C_1-C_3 alkyl group or C_1-C_3 haloalkyl group;

R^5 represents a hydrogen atom, C_1-C_3 alkyl group, C_1-C_3 haloalkyl group, cyclopropyl group, vinyl group, C_2 alkynyl group, cyano group, $-C(O)R^{20}$ group, $-CO_2R^{20}$ group, $-C(O)NR^{20}R^{21}$ group, $-CHR^{16}R^{17}CN$ group, $-CR^{16}R^{17}C(O)R^{20}$ group, $-C^{16}R^{17}CO_2R^{20}$ group, $-CR^{16}R^{17}C(O)NR^{20}R^{21}$ group, $-CHR^{16}OH$ group, $-CHR^{16}OC(O)R^{20}$ group or $-OCHR^{16}OC(O)NR^{20}R^{21}$ group, or, when G represents G-2 or G-6, R^4 and R^5 may represent C=O group together with the carbon atom to which they are attached;

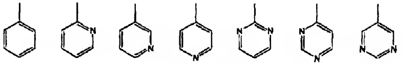
R^6 represents C_1-C_6 alkyl group, C_1-C_6 haloalkyl group, C_2-C_6 alkoxyalkyl group, C_3-C_6alkenyl group or C_3-C_6 alkynyl group;

R^7 represents a hydrogen atom, C_1-C_6 alkyl group, C_1-C_6 haloalkyl group, halogen atom, -$S(O)_2(C_1$-C_6 alkyl) group or -$C(=O)R^{22}$ group;

R^8 represents a hydrogen atom, C_1-C_8 alkyl group, C_3-C_8 cycloalkyl group, C_3-C_8 alkenyl group, C_3-C_8 alkynyl group, C_1-C_8 haloalkyl group, C_2-C_8 alkoxyalkyl group, C_3-C_8 alkoxyalkoxyalkyl group, C_3-C_8 haloalkynyl group, C_3-C_8 haloalkenyl group, C_1-C_8 alkylsulfonyl group, C_1-C_8 haloalkylsulfonyl group, C_3-C_8 alkoxycarbonylalkyl group, -$S(O)_2NH(C_1$-C_8 alkyl) group, -$C(O)R^{23}$ group or benzyl group which may be substituted with R^{24} on the phenyl ring;

R^9 represents C_1-C_8 alkyl group, C_3-C_8 cycloalkyl group, C_3-C_8 alkenyl group, C_3-C_8 alkynyl group, C_1-C_8 haloalkyl group, C_2-C_8 alkoxyalkyl group, C_2-C_8 alkylthioalkyl group, C_2-C_8 alkylsulfinylalkyl group, C_2-C_8 alkylsulfonylalkyl group, C_4-C_8 alkoxyalkoxyalkyl group, C_4-C_8 cycloalkylalkyl group, C_4-C_8 cycloalkoxyalkyl group, C_4-C_8 alkenyloxyalkyl group, C_4-C_8 alkynyloxyalkyl group, C_3-C_8 haloalkoxyalkyl group, C_4-C_8 haloalkenyloxyalkyl group, C_4-C_8 haloalkynyloxyalkyl group, C_4-C_8 cycloalkylthioalkyl group, C_4-C_8 alkenylthioalkyl group, C_4-C_8 alkynylthioalkyl group, C_1-C_4 alkyl group substituted with a phenoxy group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_3 alkyl group and C_1-C_3 haloalkyl group, C_1-C_4 alkyl group substituted with a benzyloxy group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_3 alkyl group and C_1-C_3 haloalkyl group, C_4-C_8 trialkylsyrylalkyl group, C_2-C_8 cyanoalkyl group, C_3-C_8 halocycloalkyl group, C_3-C_8 haloalkenyl group, C_5-C_8 alkoxyalkenyl group, C_5-C_8 haloalkoxyalkenyl group, C_5-C_8 alkylthioalkenyl group, C_3-C_8 haloalkynyl group, C_5-C_8 alkoxyalkynyl group, C_5-C_8 haloalkoxyalkynyl group, C_5-C_8 alkylthioalkynyl group, C_2-C_8 alkylcarbonyl group, benzyl group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_3 alkyl group, C_1-C_3

haloalkyl group, $-OR^{28}$ group, $-NR^{11}R^{28}$ group, $-SR^{28}$ group, cyano group, $-CO_2R^{28}$ group and nitro group, $-CR^{16}R^{17}COR^{10}$ group, $-CR^{16}R^{17}CO_2R^{20}$ group, $-CR^{16}R^{17}P(O)(OR^{10})_2$ group, $-CR^{16}R^{17}P(S)(OR^{10})_2$ group, $-CR^{16}R^{17}C(O)NR^{11}R^{12}$ group, $-CR^{16}R^{17}C(O)NH_2$ group, $-C(=CR^{26}R^{27})COR^{10}$ group, $-C(=CR^{26}R^{27})CO_2R^{20}$ group, $-C(=CR^{26}R^{27})P(O)(OR^{10})_2$ group, $-C(=CR^{26}R^{27})P(S)(OR^{10})_2$ group, $-C(=CR^{26}R^{27})C(O)NR^{11}R^{12}$ group, $-C(=CR^{26}R^{27})C(O)NH_2$ group, or any one of rings shown in Q-1 to Q-7:



Q-1 Q-2 Q-3 Q-4 Q-5 Q-6 Q-7

which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_6 alkyl group, C_1-C_6 haloalkyl group, C_2-C_6 alkenyl group, C_2-C_6 haloalkenyl group, C_2-C_6 alkynyl group, C_3-C_6 haloalkynyl group, C_2-C_8 alkoxyalkyl group, $-OR^{28}$ group, $-SR^{28}$ group, $-NR^{11}R^{28}$ group, C_3-C_8 alkoxycarbonylalkyl group, C_2-C_4 carboxyalkyl group, $-CO_2R^{28}$ group and cyano group;

R^{10} represents a C_1-C_6 alkyl group, C_2-C_6 alkenyl group, C_3-C_6 alkynyl group or tetrahydrofuranyl group;

R^{11} and R^{13} independently represent a hydrogen atom or C_1-C_4 alkyl group;

R^{12} represents C_1-C_6 alkyl group, C_3-C_6 cycloalkyl group, C_3-C_6 alkenyl group, C_3-C_6 alkynyl group, C_2-C_6 alkoxyalkyl group, C_1-C_6 haloalkyl group, C_3-C_6 haloalkenyl group, C_3-C_6 haloalkynyl group, phenyl group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_4 alkyl group and C_1-C_4 alkoxy group or $-CR^{16}R^{17}CO_2R^{25}$ group; or,

R^{11} and R^{12} together may represent $-(CH_2)_5$-, $-(CH_2)_4$- or $-CH_2CH_2OCH_2CH_2$-, or in that case the resulting ring may be substituted with a substituent selected from a C_1-C_3

alkyl group, a phenyl group and benzyl group;

R^{14} represents a C_1-C_4 alkyl group or phenyl group which may be substituted on the ring with a substituent selected from a halogen atom, C_1-C_3 alkyl group and C_1-C_3 haloalkyl group; or,

R^{13} and R^{14} may represent C_3-C_8 cycloalkyl group together with the carbon atom to which they are attached;

R^{15} represents C_1-C_4 alkyl group, C_1-C_4 haloalkyl group or C_3-C_6 alkenyl group;

R^{16} and R^{17} independently represent a hydrogen atom or C_1-C_4 alkyl group, C_1-C_4 haloalkyl group, C_2-C_4 alkenyl group, C_2-C_4 haloalkenyl group, C_2-C_4 alkynyl group, C_3-C_4 haloalkynyl group; or,

R^{16} and R^{17} may represent C_3-C_6 cycloalkyl group with the carbon atom to which they are attached, or the ring thus formed may be substituted with at least one substituent selected from a halogen atom, a C_1-C_3 alkyl group and C_1-C_3 haloalkyl group;

R^{18} represents a hydrogen atom, C_1-C_6 alkyl group, C_3-C_6 alkenyl group or C_3-C_6 alkynyl group;

R^{19} represents a hydrogen atom, C_1-C_4 alkyl group or halogen atom,

R^{20} represents a hydrogen atom, C_1-C_6 alkyl group, C_3-C_6 cycloalkyl group, C_3-C_6 alkenyl group, C_3-C_6 alkynyl group, C_2-C_6 alkoxyalkyl group, C_1-C_6 haloalkyl group, C_3-C_6 haloalkenyl group, C_3-C_6 haloalkynyl group, phenyl group which may be substituted on the ring with at least one substituent selected from a halogen atom, C_1-C_4 alkyl group and $-OR^{28}$ group, or $-CR^{16}R^{17}CO_2R^{25}$ group;

R^{21} represents a hydrogen atom, C_1-C_2 alkyl group or $-CO_2(C_1$-C_4 alkyl) group;

R^{22} represents a hydrogen atom, C_1-C_6 alkyl group, C_1-C_6 alkoxy group or $NH(C_1$-C_6 alkyl) group;

R^{23} represents C_1-C_6 alkyl group, C_1-C_6 haloalkyl group, C_1-C_6 alkoxy group,

$NH(C_1\text{-}C_6$ alkyl) group, benzyl group, $C_2\text{-}C_8$ dialkylamino group or phenyl group which may be substituted with R^{24};

R^{24} represents $C_1\text{-}C_6$ alkyl group, 1 to 2 halogen atoms, $C_1\text{-}C_6$ alkoxy group or CF_3 group;

R^{25} represents $C_1\text{-}C_6$ alkyl group, $C_1\text{-}C_6$ haloalkyl group, $C_3\text{-}C_6$ alkenyl group, $C_3\text{-}C_6$ haloalkenyl group, $C_3\text{-}C_6$ alkynyl group or $C_3\text{-}C_6$ haloalkynyl group;

R^{26} and R^{27} each represent independently a hydrogen atom, $C_1\text{-}C_4$ alkyl group, $C_1\text{-}C_4$ haloalkyl group, $C_2\text{-}C_4$ alkenyl group, $C_2\text{-}C_4$ haloalkenyl group, $C_2\text{-}C_4$ alkynyl group, $C_3\text{-}C_4$ haloalkynyl group, $-OR^{28}$ group, $-NHR^{28}$ group, or $-SR^{28}$ group; or,

R^{26} and R^{27} may represent $C_3\text{-}C_8$ cycloalkyl group with the carbon atom to which they are attached, or each of the ring thus formed may be substituted with at least one substituent selected from a halogen atom, $C_1\text{-}C_3$ alkyl group and $C_1\text{-}C_3$ haloalkyl group; and,

R^{28} represents a hydrogen atom, $C_1\text{-}C_6$ alkyl group, $C_1\text{-}C_6$ haloalkyl group, $C_3\text{-}C_6$ alkenyl group, $C_3\text{-}C_6$ haloalkenyl group, $C_3\text{-}C_6$ alkynyl group, $C_3\text{-}C_6$ haloalkynyl group, $C_2\text{-}C_4$ carboxyalkyl group, $C_3\text{-}C_8$ alkoxycarbonylalkyl group, $C_3\text{-}C_8$ haloalkoxycarbonylalkyl group, $C_5\text{-}C_9$ alkenyloxycabonylalkyl group, $C_5\text{-}C_9$ haloalkenyloxycabonylalkyl group, $C_5\text{-}C_9$ alkynyloxycabonylalkyl group, $C_5\text{-}C_9$ haloalkynyloxycabonylalkyl group, $C_5\text{-}C_9$ cycloalkoxycabonylalkyl group or $C_5\text{-}C_9$ halocycloalkoxycabonylalkyl group.

39. A method of controlling weeds comprising a step of applying a compound to a cultivation area of a plant expressing at least one protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224;

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(A28) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA

amplifiable by a polymerase chain reaction with a primer comprising the nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, a primer comprising the nucleotide sequence shown in SEQ ID NO: 129 and as a template a chromosomal DNA of Streptomyces phaeochromogenes, Streptomyces testaceus, Streptomyces achromogenes, Streptomyces griseofuscus, Streptomyces thermocoerulescens, Streptomyces nogalater, Streptomyces tsusimaensis, Streptomyces glomerochromogenes, Streptomyces olivochromogenes, Streptomyces ornatus, Streptomyces griseus, Streptomyces lanatus, Streptomyces misawanensis, Streptomyces pallidus, Streptomyces roseorubens, Streptomyces rutgersensis, Streptomyces steffisburgensis or Saccharopolyspora taberi.

40. A method of evaluating the resistance of a cell to a compound of formula (I), said method comprising:

(3) a step of contacting said compound with a cell expressing at least one herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system

containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID

NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(4) a step of evaluating the degree of damage to the cell which contacted the compound in the above step (1).

41. The method according to claim 40, wherein the cell is a microorganism cell or plaint cell.

42. A method of selecting a cell resistant to a compound of formula (I), said method comprising a step of selecting a cell based on the resistance evaluated in the method according to claim 40.

43. The cell resisntant to herbicide selected by the method according to claim 42, or the culture thereof, .

44. A method of evaluating the resistance of a plant to a compound of formula (I), said method comprising:

(3) a step of contacting said compound with a plant expressing at least one herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula

(III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequences shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide

sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224; and

(4) a step of evaluating the degree of damage to the plant which contacted the compound described in step (1).

45. A method of selecting a plant resistant to a compound of formula (I), said method comprising a step of selecting a plant based on the resistance evaluated in the method according to claim 44.

46. A herbicidally resistant plant selected from the method according to claim 45, or the progeny thereof.

47. A method of treating a compound of formula (I), said method comprising reacting said compound in the presence of an electron transport system containing an electron donor, with at least one herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system

containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO. 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of

formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224.

48. The method according to claim 47, wherein reacting the compound with the herbicide metabolizing protein by contacting the compound with a transformant in which a DNA encoding the herbicide metabolizing protein is introduced into a host cell in a position enabling its expression in said cell.

49. Use for treating the compound of formula (I) of a herbicide metabolizing protein selected from the group consisting of:

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system

containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding any one of the amino acid sequences shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding the amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID

NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224.

50. Use for treating a compound of formula (I) of a polynucleotide encoding a herbicide metabolizing protein selected from the group consisting of

(A1) a protein comprising the amino acid sequence shown in SEQ ID NO: 1;

(A2) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(A3) a protein comprising the amino acid sequence shown in SEQ ID NO: 3;

(A4) a protein comprising the amino acid sequence shown in SEQ ID NO: 108;

(A5) a protein having an ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III) and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A6) a protein having an ability to convert in the presence of an electron transport system containing an electron donor a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 80% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 108;

(A7) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA that hybridizes, under stringent conditions, to a DNA comprising a nucleotide sequence encoding an amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID

NO: 3 or SEQ ID NO: 108;

(A8) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a DNA amplifiable by a polymerase chain reaction with a primer comprising a nucleotide sequence shown in SEQ ID NO: 129, a primer comprising a nucleotide sequence shown in any one of SEQ ID NOs: 124 to 128, and as a template a chromosome of a microorganism belonging to Streptomyces or Saccharopolyspora;

(A9) a protein comprising an amino acid sequence shown in SEQ ID NO: 4;

(A11) a protein comprising the amino acid sequence shown in SEQ ID NO: 159;

(A12) a protein comprising the amino acid sequence shown in SEQ ID NO: 160;

(A13) a protein comprising the amino acid sequence shown in SEQ ID NO: 136;

(A14) a protein comprising the amino acid sequence shown in SEQ ID NO: 137;

(A15) a protein comprising the amino acid sequence shown in SEQ ID NO: 138;

(A16) a protein comprising the amino acid sequence shown in SEQ ID NO: 215;

(A17) a protein comprising the amino acid sequence shown in SEQ ID NO: 216;

(A18) a protein comprising the amino acid sequence shown in SEQ ID NO: 217;

(A19) a protein comprising the amino acid sequence shown in SEQ ID NO: 218;

(A20) a protein comprising the amino acid sequence shown in SEQ ID NO: 219;

(A21) a protein comprising the amino acid sequence shown in SEQ ID NO: 220;

(A22) a protein comprising the amino acid sequence shown in SEQ ID NO: 221;

(A23) a protein comprising the amino acid sequence shown in SEQ ID NO: 222;

(A24) a protein comprising the amino acid sequence shown in SEQ ID NO: 223;

(A25) a protein comprising the amino acid sequence shown in SEQ ID NO: 224;

(A26) a protein comprising an ability to convert in the presence of an electron transport

system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221 or SEQ ID NO: 223 or an amino acid sequence having at least 90% sequence identity with an amino acid sequence shown in any one of SEQ ID NO: 160, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 222 or SEQ ID NO: 224; and

(A27) a protein having the ability to convert in the presence of an electron transport system containing an electron donor, a compound of formula (II) to a compound of formula (III), and comprising an amino acid sequence encoded by a nucleotide sequence having at least 90% sequence identity with a nucleotide sequence encoding an amino acid sequence shown in any one of SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO:218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223 or SEQ ID NO: 224.

Application number / numéro de demande: JP02/10789

Figures: 26

Pages:

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au 10ème étage)

DRAWINGS

Fig. 1

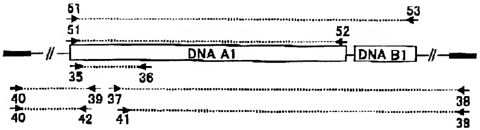


Fig. 2

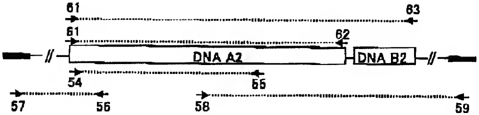
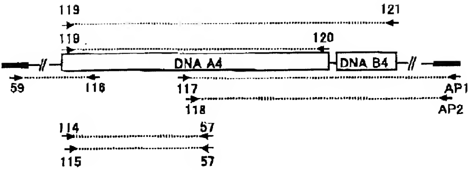


Fig. 3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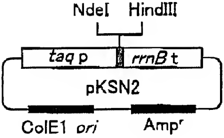
121
119
120
DNA A4
DNA B4
59
116
117
AP1
118
AP2
114
57
115
57

Fig. 4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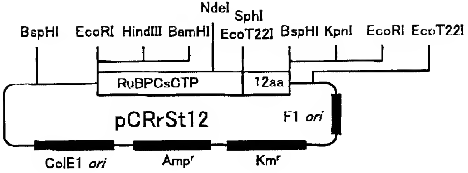
HindIII
taq p
rrnB t
pKSN2
ColE1 ori
Amp^r

Fig. 5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HindIII
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BspHI
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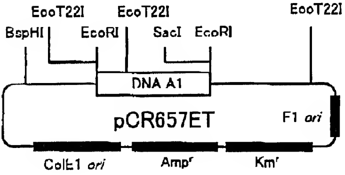
12aa
pCRrSt12
F1 *ori*
ColE1 *ori*
Amp^r
Km^r

Fig. 6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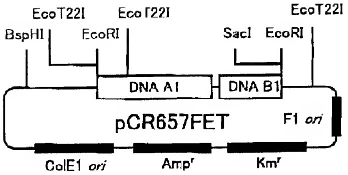
EcoT22I
EcoT22I
BspHI
EcoRI
SacI
EcoRI
DNA A1
pCR657ET
F1 *ori*
ColE1 *ori*
Amp^r
Km^r

Fig 7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EcoT22I
BspHI
EcoRI
SacI
EcoRI
DNA A1
DNA B1
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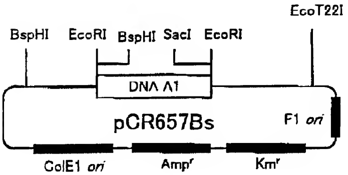
F1 ori
ColE1 ori
Amp^r
Km^r

Fig. 8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BspHI
SacI
EcoRI
DNA A1
pCR657Bs
F1 ori
ColE1 ori
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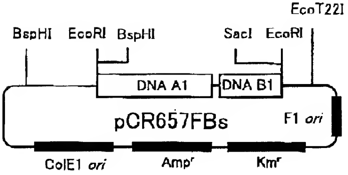
Km^r

Fig. 9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EcoRI
BspHI
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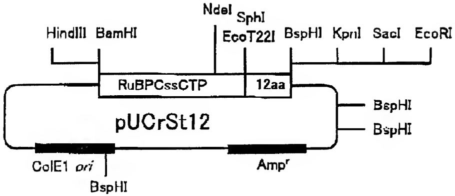
EcoRI
DNA A1
DNA B1
pCR657FBs
F1 *ori*
ColE1 *ori*
Amp^r
Km^r

Fig. 10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EcoT22I
BspHI
KpnI
SacI
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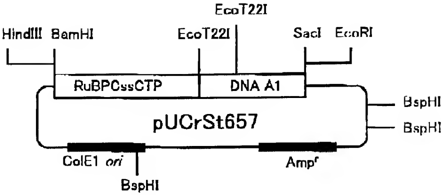
BspHI
BspHI
ColE1 *ori*
BspHI
Amp^r

Fig. 11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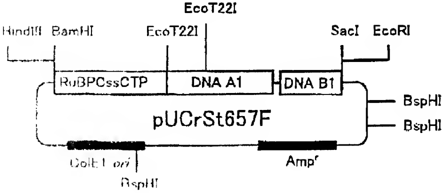
ColE1 ori
Ampr
BspHI

Fig. 12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EcoT22I
SacI
EcoRI
RuBPCssCTP
DNA A1
DNA B1
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BspHI
pUCrSt657F
ColE1 ori
Ampr
BspHI

Fig. 13

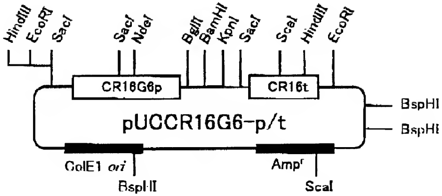

Fig. 14

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CGCCGGCGCTTAA

Fig. 15

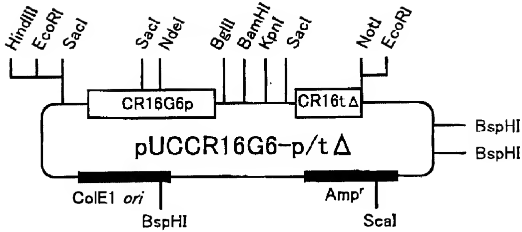


Fig. 16

AGCTTGCGGCCGC
ACGCCGGCGAT

Fig. 17

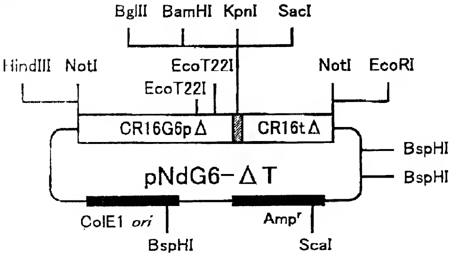
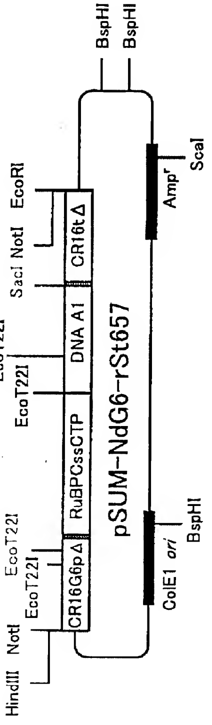


Fig. 18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EcoT22I
CR16G6pΔ
RuBPCssCTP
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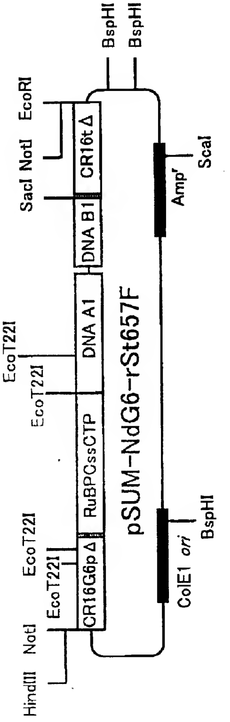
EcoT22I
DNA A1
SacI
NotI
EcoRI
CR16tΔ
BspHI
BspHI
ScaI
Amp^r
ColE1 ori
BspHI

Fig. 19



pSUM-NdG6-rSt657F
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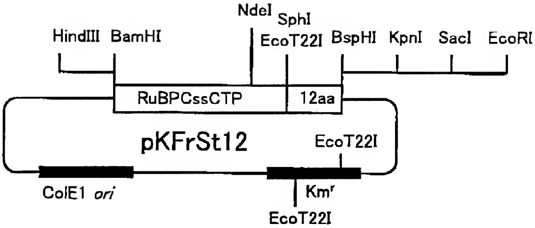
NotI
EcoT22I
EcoT22I
CR16G6pΔ
RuBPCssCTP
EcoT22I
EcoT22I
DNA A1
DNA B1
SacI
NotI
EcoRI
CR16tΔ
BspHI
BspHI
Amp^r
ScaI
ColE1 ori
BspHI

Fig. 20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SphI
HindIII
BamHI
EcoT22I
BspHI
KpnI
SacI
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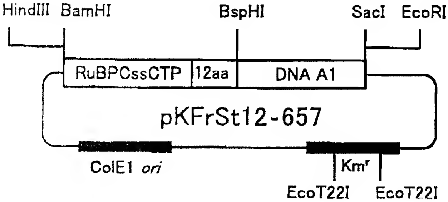
RuBPCssCTP
12aa
pKFrSt12
EcoT22I
ColE1 ori
Km^r
EcoT22I

Fig. 21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BspHI
SacI
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12aa
DNA A1
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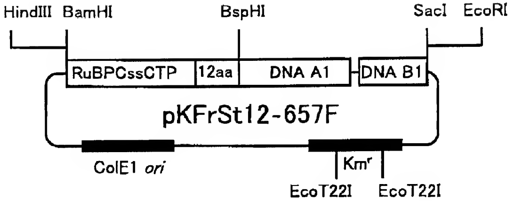
ColE1 ori
Km^r
EcoT22I
EcoT22I

Fig. 22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BamHI
BspHI
SacI
EcoRI
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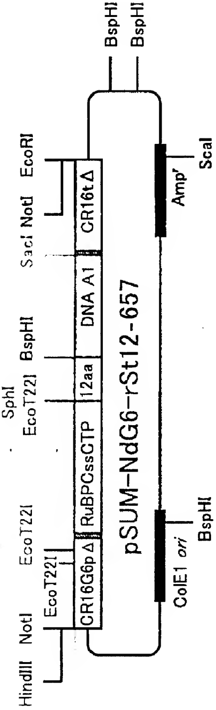
12aa
DNA A1
DNA B1
pKFrSt12-657F
ColE1 ori
Km^r
EcoT22I
EcoT22I

Fig. 23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ScaI
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12aa
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EcoT22I
RuBPCssCTP
pSUM-NdG6-rSt12-657
EcoT22I
EcoT22I
CR16G6pΔ
ColE1 ori
BspHI
HindIII
NotI

Fig. 24

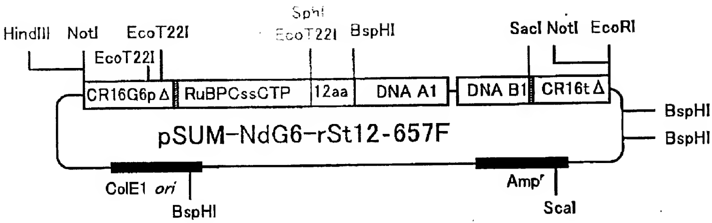
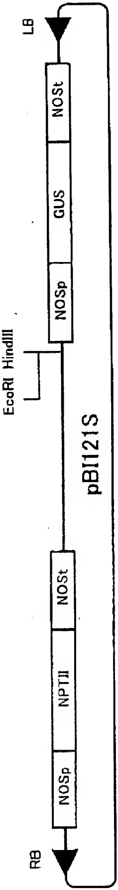


Fig. 25

AGCTTGCGGCCGCGAATTC
ACGCCGGCGCTTAAGTCGA

Fig. 26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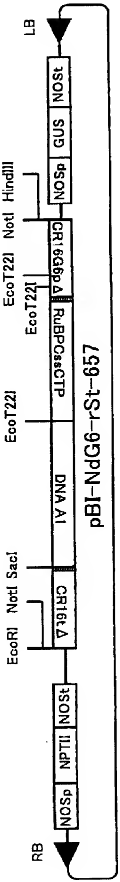
GUS
NOSp
EcoRI HindIII
pBI121S
NOSt
NPTII
NOSp
RB

Fig. 27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NOSp
NotI HindIII
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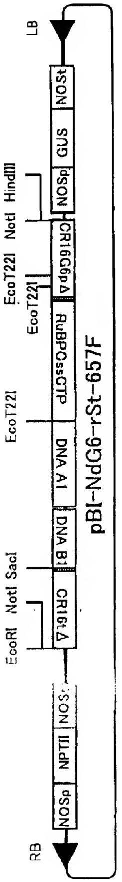
RB
pBI-NdG6-rSt-657

Fig. 28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HindIII
NotI
EcoT22I
CR16G6pΔ
EcoT22I
RuBPCssCTP
EcoT22I
DNA A1
DNA B1
SacI
NotI
EcoRI
CR16tΔ
NOSt
NPTII
NOSp
RB
pBI-NdG6-rSt-657F

Fig. 29

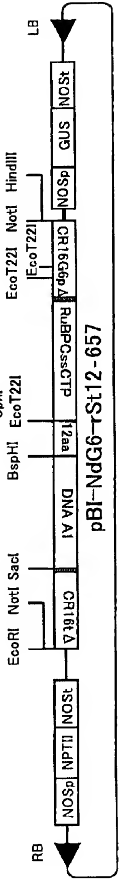

LB
NOSt
GUS
NOSp
HindIII
NotI
EcoT22I
EcoT22I
CR16G6pΔ
RuBPCssCTP
SphI
EcoT22I
12aa
BspHI
DNA A1
SacI
NotI
EcoRI
CR16tΔ
NOSt
NPTII
NOSp
RB
pBI-NdG6-rSt12-657

Fig. 30

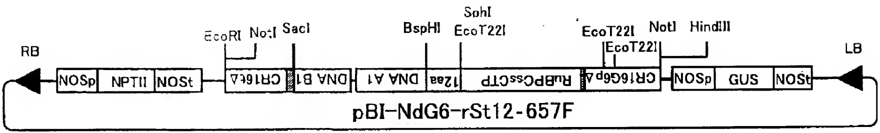

EcoRI
NotI
SacI
BspHI
SphI
EcoT22I
EcoT22I
EcoT22I
NotI
HindIII
RB
LB
NOSp
NPTII
NOSt
CR16tΔ
DNA B1
DNA A1
12aa
RuBPCssCTP
CR16G6pΔ
NOSp
GUS
NOSt
pBI-NdG6-rSt12-657F

Fig. 31

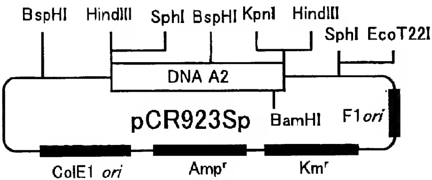

BspHI
HindIII
SphI
BspHI
KpnI
HindIII
SphI
EcoT22I
DNA A2
pCR923Sp
BamHI
F1 ori
ColE1 ori
Amp^r
Km^r

Fig. 32

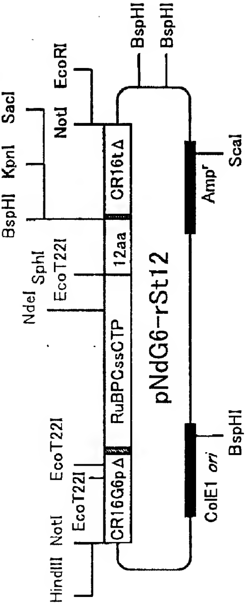

pNdG6-rSt12
HindIII
NotI
EcoT22I
EcoT22I
EcoT22I
CR16G6pΔ
RuBPCssCTP
NdeI
SphI
EcoT22I
12aa
BspHI
KpnI
SacI
CR16tΔ
NotI
EcoRI
BspHI
BspHI
ScaI
Amp^r
ColE1 ori
BspHI

Fig. 33

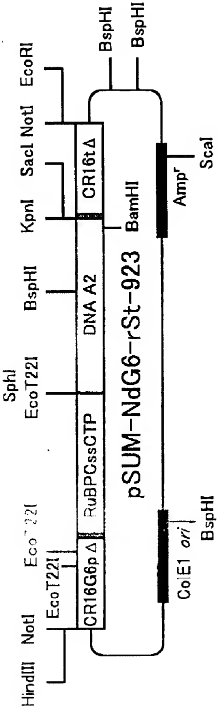
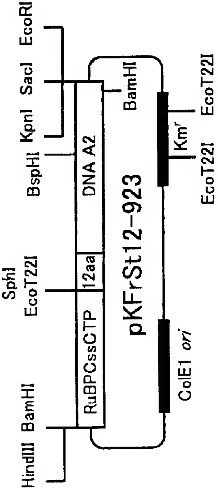

HindIII
NotI
EcoT22I
EcoT22I
SphI
EcoT22I
BspHI
KpnI
SacI
NotI
EcoRI
BspHI
BspHI
CR16G6pΔ
RuBPCssCTP
DNA A2
CR16tΔ
BamHI
pSUM-NdG6-rSt-923
Amp^r
ScaI
ColE1 ori
BspHI

Fig. 34


HindIII
BamHI
SphI
EcoT22I
BspHI
KpnI
SacI
EcoRI
RuBPCssCTP
12aa
DNA A2
BamHI
pKFrSt12-923
Km^r
EcoT22I
EcoT22I
ColE1 ori

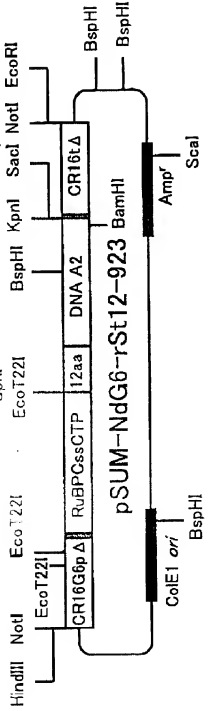

HindIII
NotI
EcoT22I
EcoT22I
CR16G6pΔ
RuBPCssCTP
SphI
EcoT22I
12aa
DNA A2
BspHI
KpnI
SacI
NotI
EcoRI
CR16tΔ
BamHI
BspHI
BspHI
pSUM-NdG6-rSt12-923
Amp^r
ScaI
ColE1 ori
BspHI

Fig. 35

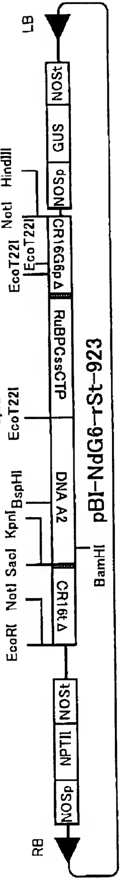

RB
NOSp
NPTII
NOSt
EcoRI
NotI
SacI
KpnI
BspHI
CR16tΔ
DNA A2
BamHI
SphI
EcoT22I
RuBPCssCTP
pBI-NdG6-rSt-923
EcoT22I
EcoT22I
CR16G6pΔ
NotI
HindIII
NOSp
GUS
NOSt
LB

Fig. 36

Fig. 37

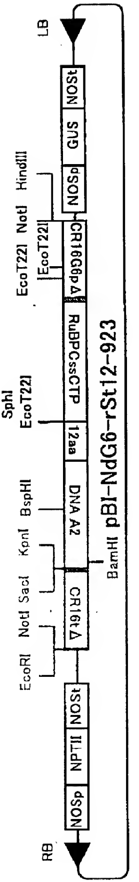

RB
NOSp
NPTII
NOSt
EcoRI
NotI
SacI
KpnI
BspHI
SphI
EcoT22I
EcoT22I
NotI
HindIII
EcoT22I
CR16tΔ
BamHI
DNA A2
12aa
RuBPCssCTP
CR16G6pΔ
NOSp
GUS
NOSt
LB
pBI-NdG6-rSt12-923

Fig. 38

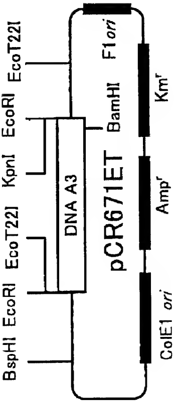
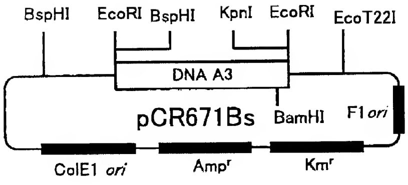
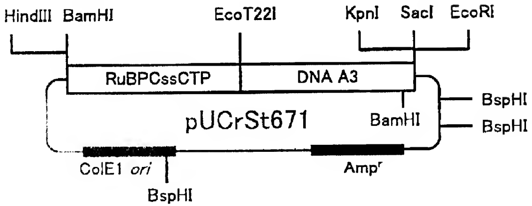

BspHI
EcoRI
EcoT22I
KpnI
EcoRI
EcoT22I
DNA A3
BamHI
F1 ori
Km^r
Amp^r
ColE1 ori
pCR671ET

Fig. 39



BspHI
EcoRI
BspHI
KpnI
EcoRI
EcoT22I
DNA A3
pCR671Bs
BamHI
F1 ori
ColE1 ori
Amp^r
Km^r

Fig. 40



HindIII
BamHI
EcoT22I
KpnI
SacI
EcoRI
RuBPCssCTP
DNA A3
BspHI
BspHI
pUCrSt671
BamHI
ColE1 ori
BspHI
Amp^r

Fig. 41

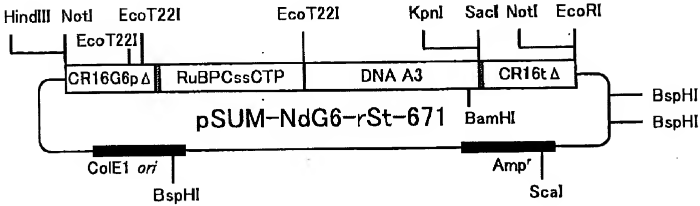

HindIII
NotI
EcoT22I
EcoT22I
EcoT22I
KpnI
SacI
NotI
EcoRI
CR16G6pΔ
RuBPCssCTP
DNA A3
CR16tΔ
BspHI
BspHI
pSUM-NdG6-rSt-671
BamHI
ColE1 ori
BspHI
Amp^r
ScaI

Fig. 42

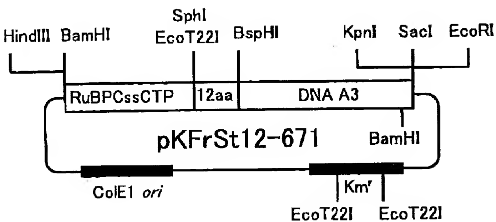

HindIII
BamHI
SphI
EcoT22I
BspHI
KpnI
SacI
EcoRI
RuBPCssCTP
12aa
DNA A3
pKFrSt12-671
BamHI
ColE1 ori
Km^r
EcoT22I
EcoT22I

Fig. 43

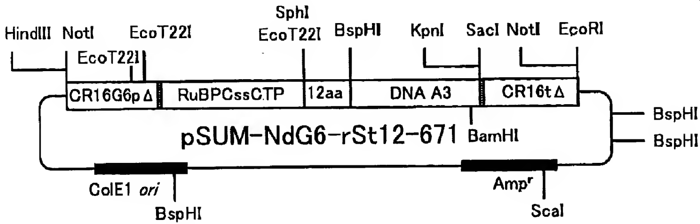

HindIII NotI
EcoT22I
EcoT22I
SphI
EcoT22I
BspHI
KpnI
SacI NotI
EcoRI
CR16G6pΔ
RuBPCssCTP
12aa
DNA A3
CR16tΔ
BspHI
BspHI
pSUM-NdG6-rSt12-671
BamHI
ColE1 ori
BspHI
Amp^r
ScaI

Fig. 44

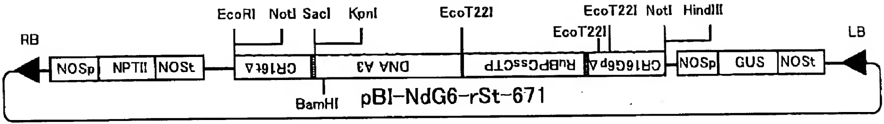
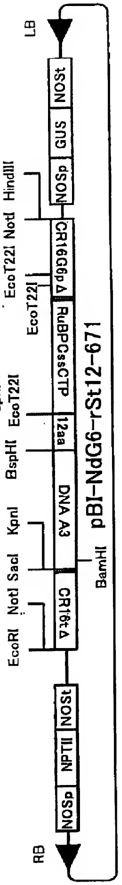

RB
NOSp
NPTII
NOSt
EcoRI NotI SacI KpnI
CR16tΔ
DNA A3
BamHI
EcoT22I
RuBPCssCTP
EcoT22I
EcoT22I NotI HindIII
CR16G6pΔ
pBI-NdG6-rSt-671
NOSp
GUS
NOSt
LB

Fig. 45



LB
NOSt
GUS
NOSp
HindIII
NotI
CR16G6pΔ
EcoT22I
EcoT22I
RuBPCssCTP
SphI
EcoT22I
12aa
BspHI
DNA A3
KpnI
SacI
BamHI
NotI
CR16tΔ
EcoRI
NOSt
NPTII
NOSp
RB
pBI-NdG6-rSt12-671

Fig. 47

AGCTATTTTTTAATAAAATCAGGAGGAAAAAACATATGAGCAAGCTTGGCTGTTTTGGCGGATGAGAGAAGA
TAAAAAATTATTTTAGTCCTCCTTTTTTGTATACTCGTTCGAACCGACAAAACCGCCTACTCTCTTCT

Fig. 48

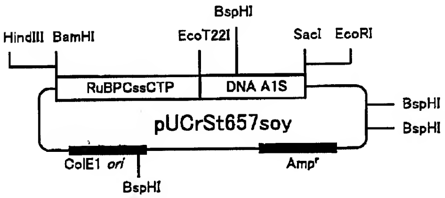


Fig. 49

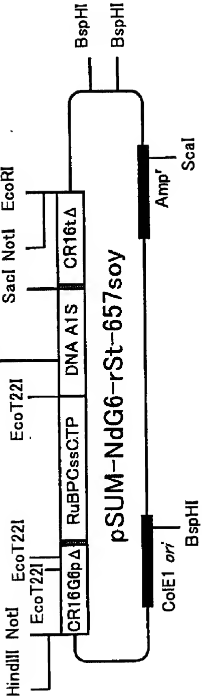

pSUM-NdG6-rSt-657soy
HindIII NotI
EcoT22I
EcoT22I
EcoT22I
BspHI
SacI NotI EcoRI
BspHI
BspHI
ScaI
CR16G6pΔ
RuBPCssCTP
DNA A1S
CR16tΔ
Amp^r
ColE1 ori
BspHI

Fig. 50

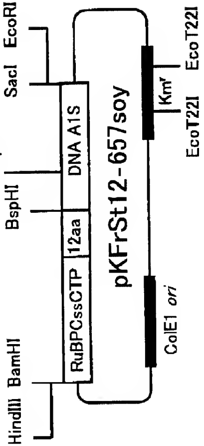

pKFrSt12-657soy
HindIII BamHI
BspHI
BspHI
SacI EcoRI
EcoT22I
EcoT22I
RuBPCssCTP
12aa
DNA A1S
Km^r
ColE1 ori

Fig. 51

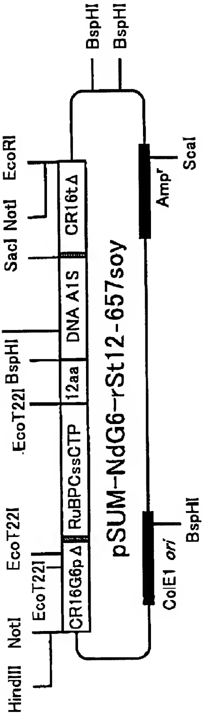

HindIII
NotI
EcoT22I
EcoT22I
EcoT22I
BspHI
BspHI
SacI NotI
EcoRI
BspHI
BspHI
CR16G6pΔ
RuBPCssCTP
12aa
DNA A1S
CR16tΔ
pSUM-NdG6-rSt12-657soy
ColE1 ori
BspHI
Amp^r
ScaI

Fig. 52

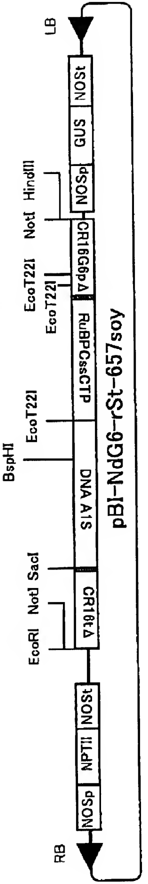

RB
NOSp
NPTII
NOSt
EcoRI
NotI
SacI
CR16tΔ
BspHI
DNA A1S
EcoT22I
RuBPCssCTP
EcoT22I
EcoT22I
CR16G6pΔ
NotI
HindIII
NOSp
GUS
NOSt
LB
pBI-NdG6-rSt-657soy

Fig. 53

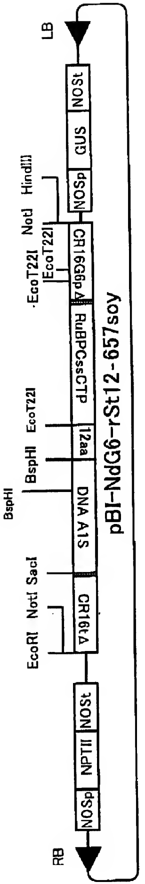

pBI-NdG6-rSt12-657soy
RB
NOSp
NPTII
NOSt
EcoRI
NotI
SacI
CR16tA
DNA A15
BspHI
BspHI
12aa
EcoT22I
RuBPCssCTP
EcoT22I
EcoT22I
CR16G6pA
NotI
HindIII
NOSp
GUS
NOSt
LB

Fig. 54

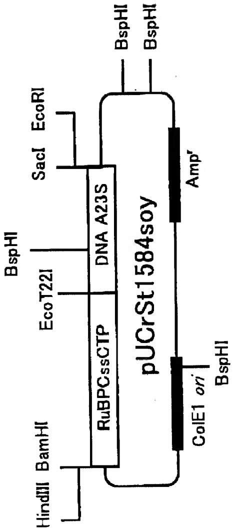

pUCrSt1584soy
HindIII
BamHI
RuBPCssCTP
EcoT22I
BspHI
DNA A23S
SacI
EcoRI
BspHI
BspHI
Amp^r
ColE1 ori
BspHI

Fig. 55

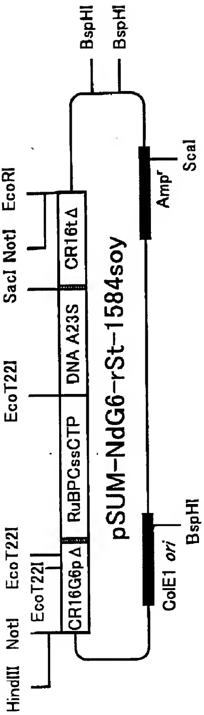

HindIII
NotI
EcoT22I
EcoT22I
CR16G6pΔ
RuBPCssCTP
EcoT22I
DNA A23S
SacI
NotI
EcoRI
CR16tΔ
pSUM-NdG6-rSt-1584soy
BspHI
BspHI
Ampr
ScaI
ColE1 ori
BspHI

Fig. 56

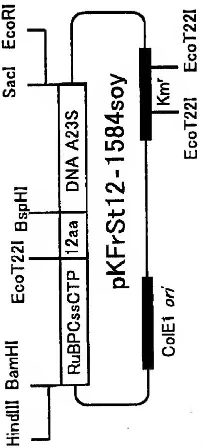

HindIII
BamHI
EcoT22I
BspHI
SacI
EcoRI
RuBPCssCTP
12aa
DNA A23S
pKFrSt12-1584soy
Kmr
EcoT22I
EcoT22I
ColE1 ori

Fig. 57

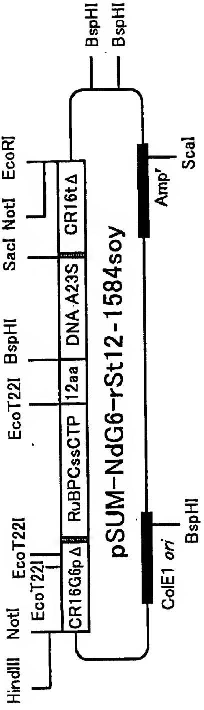

HindIII
NotI
EcoT22I
EcoT22I
EcoT22I
BspHI
SacI
NotI
EcoRI
CR16G6pΔ
RuBPCssCTP
12aa
DNA A23S
CR16tΔ
BspHI
BspHI
Amp'
ScaI
ColE1 ori
BspHI
pSUM-NdG6-rSt12-1584soy

Fig. 58

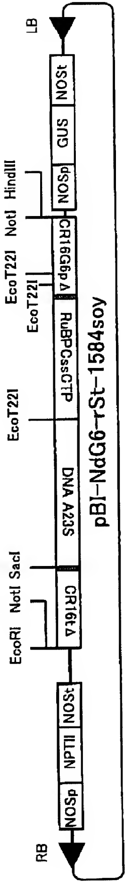

LB
NOSt
GUS
NOSp
HindIII
NotI
EcoT22I
EcoT22I
CR16G6pΔ
RuBPCssCTP
EcoT22I
DNA A23S
SacI
NotI
EcoRI
CR16tΔ
NOSt
NPTII
NOSp
RB
pBI-NdG6-rSt-1584soy

Fig. 59

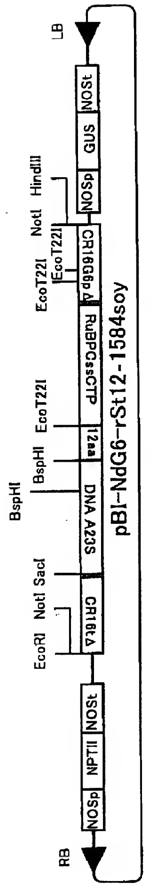

pBI-NdG6-rSt12-1584soy
RB
LB
NOSp
NPTII
NOSt
CR16tΔ
DNA A23S
12aa
RuBPCssCTP
CR16G6pΔ
NOSp
GUS
NOSt
EcoRI
NotI
SacI
BspHI
BspHI
EcoT22I
EcoT22I
EcoT22I
NotI
HindIII

Fig. 60

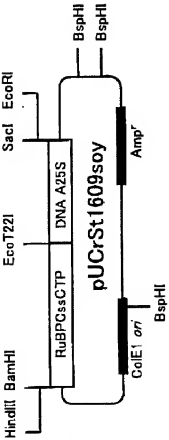

pUCrSt1609soy
HindIII
BamHI
EcoT22I
SacI
EcoRI
BspHI
BspHI
BspHI
RuBPCssCTP
DNA A25S
Amp^r
ColE1 ori

Fig. 61

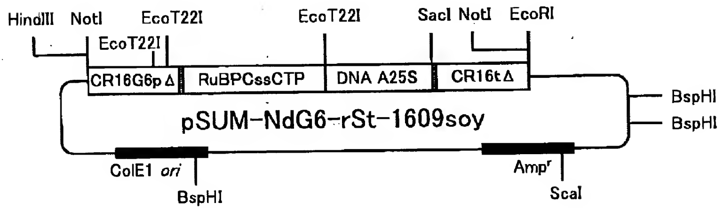


Fig. 62

TGCAGGTGTGGCCACCAATTGGCAAGAAGAAATGCA
ACGTACGTCCACACCGGTGGTTAACCGTTCTTCTTT

Fig. 63

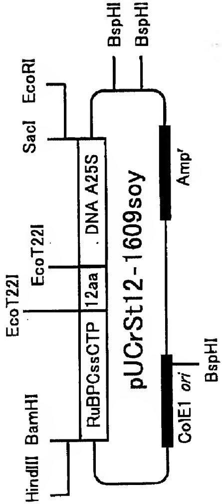
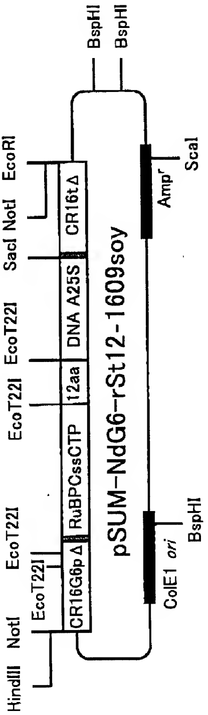

HindIII
BamHI
EcoT22I
EcoT22I
SacI
EcoRI
BspHI
BspHI
RuBPCssCTP
12aa
DNA A25S
pUCrSt12-1609soy
Amp^r
ColE1 ori
BspHI

Fig. 64


HindIII
NotI
EcoT22I
EcoT22I
EcoT22I
EcoT22I
SacI
NotI
EcoRI
BspHI
BspHI
CR16G6pΔ
RuBPCssCTP
12aa
DNA A25S
CR16tΔ
pSUM-NdG6-rSt12-1609soy
Amp^r
ScaI
ColE1 ori
BspHI

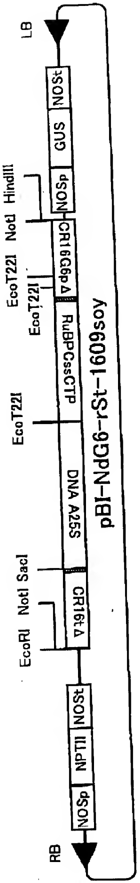

LB
NOSt
GUS
NOSp
HindIII
NotI
EcoT22I
EcoT22I
CR16G6pΔ
RuBPCssCTP
EcoT22I
DNA A25S
SacI
NotI
EcoRI
CR16tΔ
NOSt
NPTII
NOSp
RB
pBI-NdG6-rSt-1609soy

Fig. 65

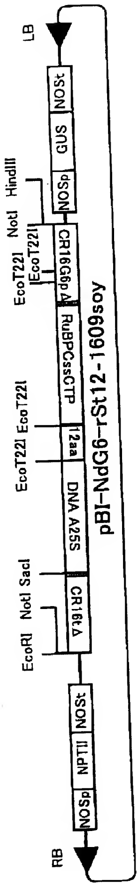

LB
NOSt
GUS
NOSp
HindIII
NotI
EcoT22I
EcoT22I
CR16G6pΔ
RuBPCssCTP
EcoT22I
EcoT22I
12aa
DNA A25S
SacI
NotI
EcoRI
CR16tΔ
NOSt
NPTII
NOSp
RB
pBI-NdG6-rSt12-1609soy

Fig. 66